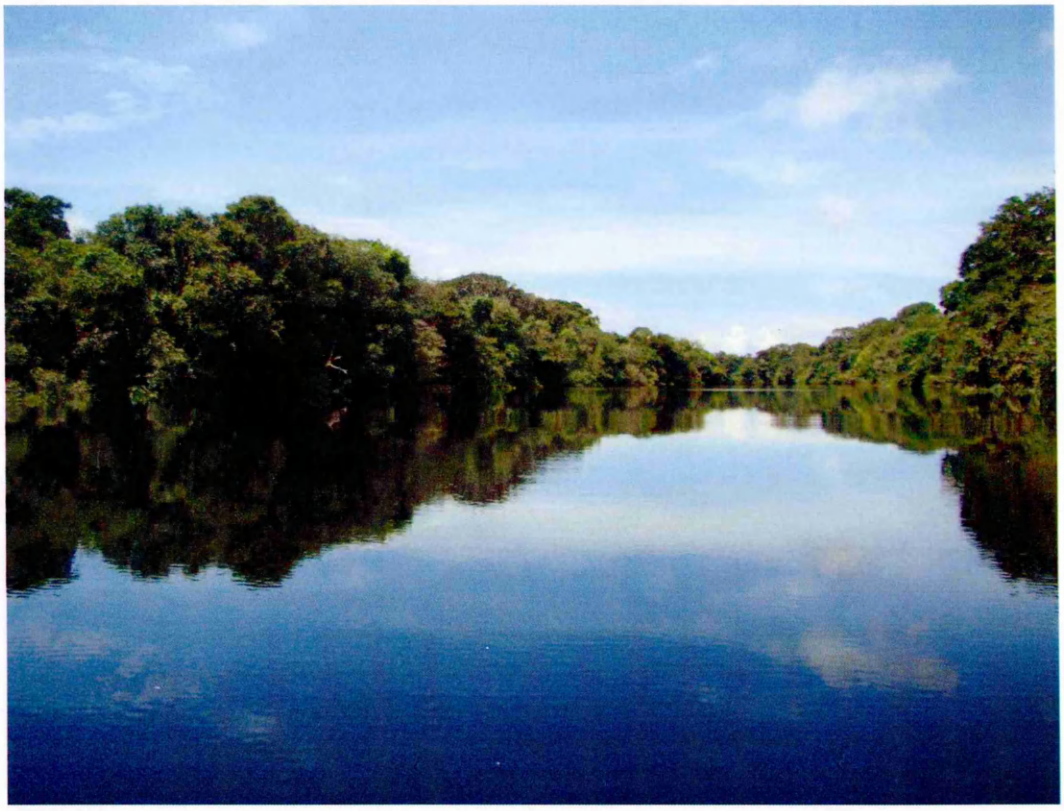
# Methane Emissions From Wetland Trees: Controls and Variability

## Thesis

# METHANE EMISSIONS FROM WETLAND TREES: CONTROLS AND VARIABILITY



**Sunitha Rao Pangala**

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**Department of Environment, Earth & Ecosystems**

**The Open University**

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# Abstract

Methane ($CH_4$) produced in wetland soil generally is thought to be released to the atmosphere primarily via diffusion, ebullition and transport through aerenchyma of herbaceous plants adapted to waterlogged soils. The role of trees as a conduit for $CH_4$ export from soil to the atmosphere has received limited attention despite laboratory studies of saplings demonstrating that wetland trees have a significant capacity to transport soil-produced $CH_4$ to the atmosphere.

In order to investigate the role of trees in transporting soil-produced $CH_4$ to the atmosphere and assess its ecosystem contributions, tree-mediated $CH_4$ flux was measured *in situ* from a temperate forested wetland (Flitwick Moor, UK) dominated by *Alnus glutinosa* and *Betula pubescens* and from a tropical forested wetland (Borneo, Indonesia). Mesocosm experiments complemented *in situ* data, in which $CH_4$ emissions were measured from *Alnus glutinosa* saplings subjected to two water-table treatments. In both the *in situ* and mesocosm studies, $CH_4$ emissions from trees were compared to $CH_4$ emissions from the soil surfaces.

Both temperate and tropical tree species released significant quantities of $CH_4$ from stem surfaces throughout the observation period. In *Alnus glutinosa*, $CH_4$ emissions from leaf surfaces were not detected and stem surfaces were the principle point of $CH_4$ egress. Stem-$CH_4$ emissions from both *Alnus glutinosa* and *Betula pubescens* were less sensitive to small changes in water-table variations when compared to $CH_4$ emissions from soil surfaces, however, the quantity, temporal variability and $CH_4$ transport mechanisms differed between the two tree species. Stem-$CH_4$ emissions were controlled by a number of factors including tree physiology, abiotic factors and gas transport mechanisms. Wetland trees contributed significantly to ecosystem $CH_4$ flux (6-87%), with tropical trees dominating ecosystem level $CH_4$ fluxes. The results demonstrate that exclusion of tree-mediated $CH_4$ emissions from flux measurement campaigns conducted in forested wetlands can significantly underestimate ecosystem-wide $CH_4$ flux.

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# Abbreviations and Symbols

| | |
|---|---|
| $CH_4$ | Methane |
| $CO_2$ | Carbon Dioxide |
| CRDLS | Cavity Ring Down Laser Spectroscopy |
| DBH | Diameter at Breast Height |
| FEP | Fluorinated ethylene propylene |
| GHG(s) | Greenhouse Gas(es) |
| HW | Mesocosm water-table treatment: water-table at the soil surface |
| ICOS | Integrated Cavity Output Spectroscopy |
| LAI | Leaf Area Index |
| LW | Mesocosm water-table treatment: water-table 25 cm below the soil surface |
| $N_2$ | Di-Nitrogen |
| $N_2O$ | Nitrous oxide |
| NEP | Net Ecosystem Production |
| NPP | Net Primary Production |
| $O_2$ | Oxygen |
| PAR | Photosynthetically Active Radiation |
| PTFE | Polytetrafluoroethylene |
| PVC | Polyvinyl chloride |
| $Q_{10}$ | Temperature coefficient |
| SE | Standard Error |
| SSSI | Site of Special Scientific Interest |

# CHAPTER ONE

# General Introduction

The research presented here investigates the role of trees in transporting soil-produced methane to the atmosphere in forested wetlands, using intensive studies conducted *in situ* and in mesocosms. This introductory chapter provides context for the research, explains the knowledge gap, presents the research objectives and outlines the thesis structure.

## 1.1. The role of wetlands in greenhouse gas emissions

Wetlands cover approximately 2.12-5.86 × $10^6$ $km^2$, *c.* 3-5% of the Earth's land area (Matthews & Fung, 1987; Prigent *et al.,* 2007), yet play a significant role in global biogeochemical cycling of the greenhouse gases, particularly methane ($CH_4$) and carbon dioxide ($CO_2$). The high water-table levels in wetlands result in anoxic conditions that favour carbon accumulation through slow organic matter decomposition and consequently $CH_4$ production and $CH_4$ release. Therefore, wetlands are the largest natural source of $CH_4$ to the atmosphere, responsible for *c.* 20-40% (100-231 Tg $CH_4$ $a^{-1}$; Table 1.1) of the global $CH_4$ budget (Denman *et. al.,* 2007).

Table 1.1: Global estimates of $CH_4$ sources and sinks.

| **Natural sources** | $CH_4$ flux (Tg $a^{-1}$)[a] | Range[b] |
|---|---|---|
| Wetlands | 174 | 100-231 |
| Termites | 22 | 20-29 |
| Oceans | 10 | 4-15 |
| Hydrates | 5 | 4-5 |
| Geological | 9 | 4-14 |
| Wild animals | 15 | 15 |
| Wild fires | 3 | 2-5 |
| Total (natural) | 238 | 149-319 |
| **Anthropogenic sources** | | |
| Coal mining | 36 | 30-46 |
| Gas, oil, industry | 61 | 52-68 |
| Landfills and waste | 54 | 35-69 |
| Ruminants | 84 | 76-92 |
| Rice agriculture | 5 | 31-83 |
| Biomass burning | 47 | 14-88 |
| Total (anthropogenic) | 336 | 238-46 |
| Total (all sources) | 574 | 387-765 |
| **Sinks** | | |
| Soils | -30 | 26-34 |
| Tropospheric OH | -467 | 428-507 |
| Stratospheric loss | -39 | 30-45 |
| Total sinks | -536 | 484-586 |

[a]; Values represent the mean of the eight separate studies provided in Denman *et al.* (2007). [b]; Range is derived by Reay *et al.* (2010) from values given in Denman *et al.* (2007).

Methane has received global research focus since the 1970s (Ehhalt, 1974) due to its high global warming potential (25-33 times that of $CO_2$ in a 100-year timeframe; Forster *et al.,* 2007; Shindell *et al.,* 2009), short life time (*c.* 10-12 yrs; Forster *et al.,* 2007), accelerated increase in $CH_4$ mixing ratio post-industrialisation (160%; Etheridge *et al.,* 1998) and chemically active properties (Cicerone & Oremland, 1998). In recent years, $CH_4$ has received renewed research interest due to the instability in annual growth rate since 1980s and recent inter-annual variations (Bousquet *et al.,* 2006; Dlugokencky *et al.,* 2009). Although the reason for $CH_4$ fluctuations post-1990s is still debated (Aydin *et al.,* 2011; Dlugokencky *et al.,* 2011; Heimann, 2011; Kai *et al.,* 2011; Rigby *et al.,* 2012), recent reports suggest, among other factors, wetlands to play a pivotal role in the recent $CH_4$ growth rate (Dlugokencky *et al.,* 2009). For these reasons, an improved understanding is required of the potential for wetland ecosystems to act as sources and sinks of $CH_4$ and their response to changing climate.

### 1.2. Wetland $CH_4$ production and emission

In wetland ecosystems, conditions such as depleted dissolved $O_2$ and other electron acceptors, negative redox potential and water saturated conditions favour the growth of $CH_4$-producing archaea (methanogens) and the production of $CH_4$. Methane emission from wetlands is the net outcome of $CH_4$ production by methanogens (de-carboxylation of acetate and reduction of $CO_2$; Conrad, 1989; Whalen, 2005) and $CH_4$ oxidation by methanotrophs in the oxidised zones (rhizosphere and soil water interface; LeMer & Roger, 2001). Methane is produced by three groups of methanogens: methylotrophic, aceticlastic and $CO_2$-reducers (Boone *et al.,* 1993), as a terminal step in the complex successive anaerobic organic-matter degradation pathway, in which complex organic molecules are broken down into simpler compounds (Fig. 1.1; Schütz *et al.,* 1991; Conrad,

1993; LeMer & Roger, 2001). The $CH_4$ produced is consumed in the aerobic sections of the soil by methanotrophs (Whalen & Reeburgh, 1992; Conrad, 1993; Chan & Parkin, 2001). Other methanotrophs consume $CH_4$ already present in the atmosphere. These two types of methanotrophs are called low-affinity and high-affinity methanotrophic bacteria, respectively (Conrad, 1984; Bender & Conrad, 1993). Around 20-100% of $CH_4$ produced in wetland soil is estimated to be consumed by methanotrophs (King, 1990; Reeburgh *et al.,* 1994; Le Mer & Roger, 2001) and these microorganisms consequently play a pivotal role in $CH_4$ cycling in wetlands.

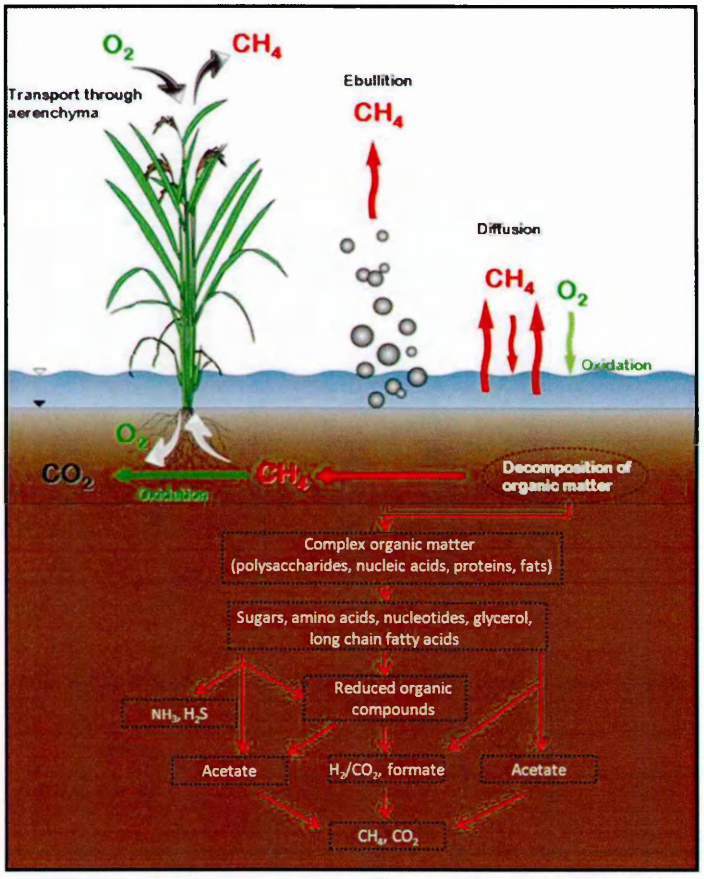


Figure 1.1: Methane production processes and emission pathways (Source: Conrad, 1993; Stams & Plugge, 2010).

Methane emissions from wetlands are characterised by high spatial and temporal variability (Christensen *et al.,* 1995; Kutzbach *et al.,* 2004). Several factors (process-level and ecosystem-level factors) exert control on $CH_4$ production and consumption (Walter *et al.,* 1996; Whalen, 2005; Conrad, 2007, 2009). Three factors: temperature, water-table depth and substrate supply are known to be the key determinant of $CH_4$ emissions from wetlands (MacDonald *et al.,* 1998; Joabsson & Christensen, 2001; Christensen *et al.,* 2003; Ström *et al.,* 2003). However, due to the extensive research on wetland $CH_4$ emissions, other environmental factors continue to be added to the list (Fig. 1.2; e.g., van Bodegom *et al.,* 2001; Le Mer & Roger, 2001; Conrad, 2007; von Fischer *et al.,* 2010).

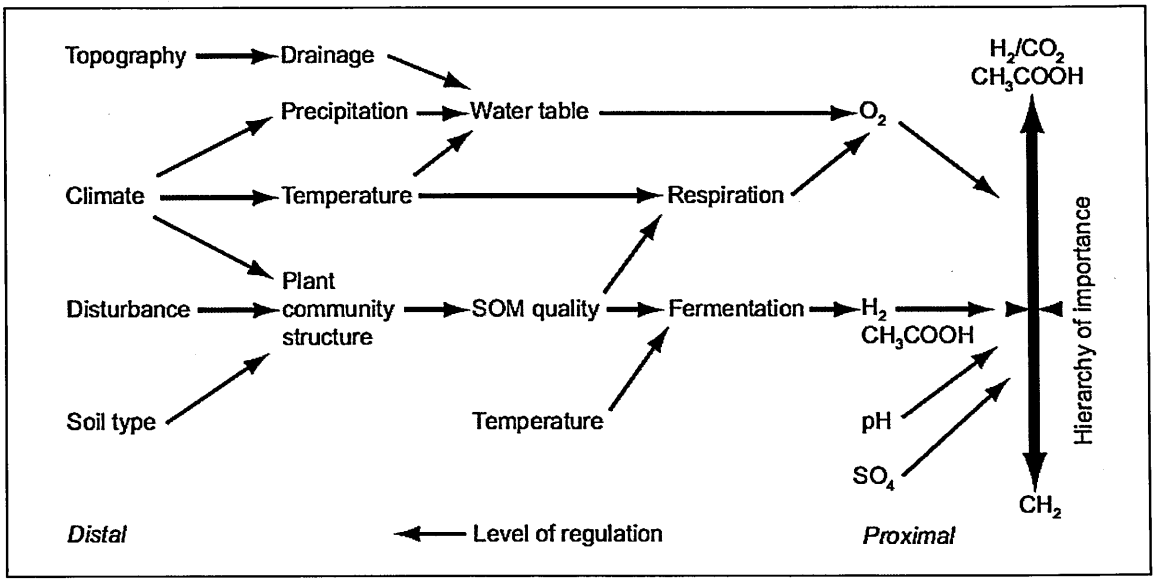


Figure 1.2: Controls on $CH_4$ production in wetland. (Source: Christensen, 2010).

### 1.3. Wetland $CH_4$ emission estimate

Wetland environments include floodplains, swamps, marshes, fens, bogs and open waters (e.g., lakes, rivers and reservoirs) and are distributed from the tropics to the poles and from the high altitude plateaus to low-lying coastal areas. Peatlands are special type of wetland, containing at least 40 cm of accumulated organic soil (Avery, 1980). Estimation of wetland distribution and $CH_4$ emissions pose technical challenges because of the diversity of wetland types. Over the last 20 years, great effort has been made to compile information on wetland distribution (e.g., Matthews & Fung, 1987; Stillwell-Soller *et al.,* 1995; Prigent *et al.,* 2001, 2007) but the inventories are surprisingly incomplete and may severely underestimate wetland area (Frey & Smith, 2007). Similarly, estimation of $CH_4$ emissions from wetlands, as well as other sources is also incomplete, resulting in significant uncertainties in the exact magnitude of each identified source and sink (Frankenberg *et al.,* 2005, 2008; do Carmo *et al.,* 2006). The most recent example of such uncertainty is $CH_4$ source strength from tropical forested regions, an area that is thought to contribute most to the total wetland annual $CH_4$ flux (*c.* 60%; Bartlett & Harriss, 1993; Bloom *et al.,* 2010).

Tropical forests attracted global research interest because recent field measurements (do Carmo *et al.,* 2006), air borne observations (Miller *et al.,* 2007), satellite observations (Frankenberg *et al.,* 2005, 2006), and inverse models (Mikaloff Fletcher *et al.,* 2004) all indicated that the size of the tropical $CH_4$ source is greater than previously thought - exceeding earlier estimates by more than 30-45 Tg (i.e. 4–9%; Frankenberg *et al.,* 2005, 2008, 2011). This discrepancy may be due to inaccurate measurement of tropical $CH_4$ emissions (e.g., Frankenberg *et al.,* 2005, 2008), previously unknown $CH_4$ sources (e.g., Keppler *et al.,* 2006; Martinson *et al.,* 2010; Covey *et al.,* 2012) and unaccounted $CH_4$ transport pathways (e.g., Rusch & Rennenberg, 1998; Rice *et al.,* 2010). These possibilities are discussed further in the following sections.

### *1.3.1. Inaccurate measurements of tropical $CH_4$ emissions*

Although the discrepancies in measured and modelled $CH_4$ concentrations in the tropics may be explained by underestimations of both established sources (wetlands, biomass burning, termites and ruminants) and new sources (aerobic $CH_4$ emissions, cryptic wetlands, tree-mediated $CH_4$ emissions), the possibility of inaccurate measurements in this region cannot be ruled out. For example, the findings of Frankenberg *et al.,* (2005) was later revised in 2008 (Frankenberg *et al.,* 2008), as the former did not account for cloud interaction and retrieval errors, making earlier findings less reliable. Furthermore, ground-based measurements in the tropics are sparse, albeit precise, leading to $CH_4$ sources being poorly sampled, inadequately characterised and lacking specificity (e.g., Bartlett *et al.,* 1988; Devol & Rickey, 1990; Wassmann *et al.,* 1992; Frankenberg *et al.,* 2011). As a result, it is not surprising that when $CH_4$ estimates from tropical regions such as those in Amazonia are revised, recent estimates (Bloom *et al.,* 2010) are significantly different from the earlier estimate (Bartlett *et al.,* 1988; Melack *et al.,* 2004). Therefore further ground-based validation measurements, process-based and satellite investigations are essential in these and other tropical regions.

### *1.3.2. Methane production by vegetation*

Keppler *et al.* (2006) published the first observation of aerobic production of $CH_4$ by plants and estimated the global vegetation to release approximately 62-236 Tg $a^{-1}$. Although the exact strength of this new $CH_4$ source is constantly being revised (e.g., Kirschbaum *et al.,* 2006, Parsons *et al.,* 2006, Butenhoff & Khalil, 2007; Bloom *et al.,* 2010), after much debate, the novel source is now confirmed. For instance, studies by Dueck *et al.* (2007) and Beerling *et al.* (2008) found no emissions from plants but experimental observations (e.g., Keppler *et al.,* 2006; Wang *et al.,* 2008; McLeod *et al.,* 2008) and atmospheric measurements (e.g., Crutzen *et al.,* 2006; Miller *et al.,* 2007) observed modest yet

significant quantities of $CH_4$ from terrestrial vegetation (McLeod *et al.,* 2008). Progress has also been made to identify the mechanism (plant pectin, ascorbic acid, cellulose, lignin and protein methionine are the precursors for plant $CH_4$ production) and controls (environmental stresses such as UV irradiation, high temperature, physical injury and drought) of this novel source (e.g., Vigano *et al.,* 2008, 2009; McLeod *et al.,* 2008; Messenger *et al.,* 2009; Qaderi & Reid, 2011) but it still remains as the one of the less understood $CH_4$ sources, although this novel source is estimated to contribute only up to 1 Tg $CH_4$ $a^{-1}$ globally.

While the magnitude, mechanisms and significance of $CH_4$ formation under aerobic conditions requires further evaluation (Bruhn *et al.,* 2012), other studies conclusively demonstrate $CH_4$ uptake by methanotrophic consortium on plants (van Aken *et al.,* 2004; Raghoebarsing *et al.,* 2005; Sundqvist *et al.,* 2012) - a process that might run parallel to $CH_4$ production by vegetation, and therefore affect net $CH_4$ flux from vegetation and should be considered in future studies.

*1.3.3. Methane production and emission within living trees*

As early as 1974, $CH_4$ was reported to be produced inside the stems of living deciduous trees by anaerobic decomposition of wet wood (Zeikus & Ward, 1974). Elevated $CH_4$ concentrations in the tree trunks can occur through bacterial infection of heartwood and/or decay of heartwood caused by fungal infection, with both conditions promoting methanogenesis (Zeikus & Ward, 1974; Schink & Ward, 1984; Covey *et al.,* 2012). Given the ubiquitous nature of heart rot (Wagener & Davidson, 1954) and elevated $CH_4$ concentrations observed in the tree trunks (15,000 $\mu L\ L^{-1}$), Covey *et al.* (2012) highlighted such $CH_4$ sources to be important in upland forests. The net efflux of $CH_4$ through the plant canopy, however, still remains unknown, as eddy covariance techniques typically fail to detect low fluxes reported for this process ($52 \pm 9.5$ ng $CH_4$ $m^{-2}$ $s^{-1}$; Covey *et al.,* 2012).

Notably, studies on $CH_4$ emissions from heart rot or wet wood do not account for $CH_4$ consumption in upland trees, a process that are known to be important and potentially counter $CH_4$ emissions (Sundqvist *et al.,* 2012).

*1.3.4. Methane emissions from other sources (tank bromeliads and termite mounds)*

In non-flooded neotropical forests, assumed to be $CH_4$ sinks, Martinson *et al.* (2010) reported other wetlands within the canopy as $CH_4$ sources (e.g., leaves of bromeliads acting as anoxic microsites, also called 'canopy wetlands'). Martinson *et al.* (2010) observed bromeliad tank water to be supersaturated with $CH_4$ (*c.* 97-1243 times the atmospheric equilibrium concentration) and estimated the global source strength to be 1.2 Tg $a^{-1}$. They also highlighted that other cryptic wetlands (e.g., tree holes, ephemeral ponds, ditches and shallow depressions in soils) may constitute equally important sources of $CH_4$.

Methane emissions from termites have also been proposed to contribute to the recent tropical $CH_4$ anomaly (Frankenberg *et al.,* 2008). Lignocellulose digestion by termites has been known and extensively studied for more than 60 years (e.g., Hungate, 1946; Sugimoto *et al.,* 1998a, b; Brune, 2006; Bignell *et al.,* 2010). However, large uncertainty currently exists in termite $CH_4$ emission estimates even at an ecosystem scale due to the incomplete understanding of termite abundance, biomass, assemblage composition, number and type of species, $CH_4$ consumption rate and differences in gas emissions between species (Sugimoto *et al.,* 1998b; Brune, 2006; Bignell *et al.,* 2010).

*1.3.5. Methane production and emission from microsites*

Despite the general consensus that dry forest soils (including neotropical and tropical forests) are net $CH_4$ sinks, there is growing evidence of methanogenic activity in such soils. Methanogens in a stasis state are known to be tolerant to certain amount of $O_2$ (Kiener & Leisinger, 1983; Fetzer *et al.,* 1993) and therefore survive long periods in dry

soils (Mayer & Conrad, 1990; Ueki *et al.,* 1997), or may be confined to anoxic soil microsites (Mayer & Conrad, 1990; Chan & Parkin, 2001; Lim *et al.,* 2012), even though soils as a whole are net $CH_4$ sinks (Anderson *et al.,* 1998; Fischer & Hedin, 2002). Additionally, most dry soils are reported to switch quickly to being a $CH_4$ source within days or weeks, when conditions are more favourable for methanogens over methanotrophs (Keller *et al.,* 1993; Yavitt *et al.,* 1995; Silver *et al.,* 1999; Teh *et al.,* 2005).

### *1.3.6. Tree-mediated $CH_4$ emission pathway*

All the sources discussed above warrant further investigation, however, based on the current best estimate, individually they only make a small contribution to the global $CH_4$ budget, none is sufficient to explain, on its own, the discrepancy in top-down and bottom-up tropical $CH_4$ emission estimates. Another pathway that has been known for over a decade, but has been rarely studied is tree-mediated $CH_4$ emissions. This pathway offers a potentially straightforward explanation for the tropical $CH_4$ discrepancy because bottom-up $CH_4$ emission estimates to-date from natural ecosystems rely almost solely on ground-based emission measurements collected using soil chambers. Such enclosures exclude tall plants and trees, and may therefore have underestimated the soil-tree $CH_4$ emission route. Given that *c.* 60% of all wetlands are forested (Matthews & Fung, 1987; Prigent *et al.,* 2007), tree-mediated $CH_4$ emissions may have global implications and therefore serves as the prime motivation for this study.

Please note: In the following sections and subsequent chapters, the term 'tree-mediated $CH_4$ emissions' collectively represents $CH_4$ emissions from all surfaces of the tree (stem and leaf surfaces). Methane emissions from the stem surfaces alone are termed as 'stem-$CH_4$ emissions'. However, when emissions from the entire tree is measured with no specific information on the $CH_4$ egress points (stem or leaf surfaces), tree-mediated $CH_4$ emissions is used.

## 1.4. Overview of the existing literature on tree-mediated $CH_4$ emission pathway

It has been well known for decades that in wetlands, soil-produced $CH_4$ is released to the atmosphere via one or a combination of three main pathways: diffusion of $CH_4$ from soil-water and water-air interface, ebullition (i.e., bubble release) and herbaceous plant-mediated (aerenchymatous) transfer (Fig. 1.1); and the relative importance of these pathways is an important control on wetland $CH_4$ emissions. In some wetlands, $CH_4$ transfer through herbaceous plants is responsible for as much as 90% of the $CH_4$ released to the atmosphere (e.g., Whiting & Chanton, 1992; Shannon *et al.,* 1996). This transport pathway is known to affect the net $CH_4$ flux due to its ability to bypass oxic zones in the soil, where $CH_4$ would otherwise be oxidised (e.g., Whiting & Chanton, 1992; Kankaala *et al.,* 2005; Käki & Kankaala, 2001). However, there is growing evidence that plant-mediated $CH_4$ emission is not limited to herbaceous plants, but may also occur in woody species.

The transport and release of $CH_4$ produced in soil by methanogens via trees through transpiration stream has been discussed at numerous occasions, more so after the discovery of aerobic $CH_4$ production by vegetation and has been proposed as an alternative mechanism to explain Keppler *et al.* (2006)'s observation (e.g., Dueck *et al.,* 2007; Nisbet *et al.,* 2009; Beerling *et al.,* 2008; Vigano *et al.,* 2008). An alternative pathway of potential GHG transport from the trunks of wetland woody species, possibly via lenticels, was suggested by Schütz *et al.* (1991). The exact pathway of tree-mediated $CH_4$ emissions is yet unidentified and the $CH_4$ egress points remain elusive (e.g., stem surfaces, leaf surfaces), nevertheless, a few laboratory (Rusch & Rennenberg, 1998; Vann & Megonigal, 2003; Garnet *et al.,* 2005; Rice *et al.,* 2010; Machacova *et al.,* 2013) and *in situ* studies (Terazawa *et al.,* 2007; Gauci *et al.,* 2010) have confirmed the presence of tree-mediated $CH_4$ emissions (Table 1.2).

The first extensive study of tree-mediated $CH_4$ emission, in particular, stem-$CH_4$ emission, was conducted in 1998, which also observed $N_2O$ emissions from the stems of *Alnus glutinosa* saplings (Rusch & Rennenberg, 1998). They reported that stem-$CH_4$ emissions decreased with increasing stem height and were strongly correlated with pore-water $CH_4$ concentration (Rusch & Rennenberg, 1998). Subsequent studies using mesocosms elucidated the importance of elevated $CO_2$ concentration, water-table depth and plant physiological parameters on tree-mediated $CH_4$ emissions. Using young *Taxodium distichum*, Vann & Megonigal (2003) observed 62 and 69% increase in $CH_4$ emission rate under elevated $CO_2$ concentrations (700 ppm), in flooded (water-table 5 cm above the soil surface) and non-flooded environment (water-table 10 cm below the soil surface), respectively. Vann & Megonigal (2003) concluded that woody plants exposed to future $CO_2$-enriched atmosphere will enhance $CH_4$ emissions regardless of the water-table position. This is because they observed a tight coupling between plant and microbial activity (e.g., strong relationship between whole-plant photosynthesis, biomass, $CH_4$ production and emissions) under elevated $CO_2$ concentrations in both the water table treatments, suggesting an increase in $CH_4$ production (due to increased assimilation and rhizodeposition stimulating methanogenesis) and transport (due to increased plant biomass).

Strong positive relationships between stomatal conductance, leaf temperature and $CH_4$ emissions from young *Taxodium distichum* were observed by Garnet *et al.* (2005). They observed diurnal patterns in $CH_4$ emission from *Taxodium distichum* to be less pronounced when compared to the wetland plants *Peltandra virginica* and *Orontium aquaticum* and estimated a temperature coefficient ($Q_{10}$) of 1.57 (temperature varied from 16 to 25 °C) for $CH_4$ emissions from *Taxodium distichum* (leaf atmosphere interface). They concluded that the effect of temperature on $CH_4$ emissions was a function of diffusion. Although stomatal conductance was found to play an important role in $CH_4$ emissions, they suggested stomata

only regulate the diffusivity of $CH_4$ from the leaf interior to the atmosphere and $CH_4$ gas transport in accordance with diffusive gas transport. Notably, unlike Rusch & Rennenberg (1998), Vann & Megonigal (2003) and Garnet *et al.* (2005) measured emissions from either the entire sapling or aboveground portion of the plant, and not from the tree stem surfaces.

The bulk of the research on tree-mediated $CH_4$ emissions to date has used mesocosms, with the exception of Terazawa *et al.* (2007) and Gauci *et al.* (2010), who examined $CH_4$ release from mature trees *in situ.* Both these studies reported similar average peak stem-$CH_4$ emissions (170 $\mu g\ m^{-2}\ hr^{-1}$ vs. 110 $\mu g\ m^{-2}\ hr^{-1}$), although studies were conducted on different tree species (*Fraxinus mandshurica* vs. *Alnus glutinosa*) and at different geographical locations (Japan vs. UK). The two studies observed differences in seasonal variation, with Gauci *et al.* (2010) reporting strong seasonal variation and Terazawa *et al.* (2007) observing significant emissions even during the leafless season, albeit the observation period was non-intensive, relatively short and excluded winter months. Nonetheless, Gauci *et al.* (2010) reported the average peak stem-$CH_4$ flux from *Alnus glutinosa* to be approximately 20% of the measured soil $CH_4$ flux, while Terazawa *et al.* (2007) estimated stem-$CH_4$ flux per unit area to be equivalent to the average soil $CH_4$ flux from that ecosystem. Pore-water $CH_4$ concentrations near the surface (0-40 cm below the soil surface) were below ambient levels ($< 2\ \mu L\ L^{-1}$) possibly due to low water-table conditions (average depth < 40 cm below the soil surface) in the Terazawa *et al.* (2007) study, which led to the conclusion that high $CH_4$ concentrations in deeper groundwater ($89\text{-}454\ \mu L\ L^{-1}$) drove stem-$CH_4$ emissions.

Table 1.2: List of all studies that have investigated $CH_4$ emissions from trees.

| Literature | Geographical location | *In situ*/mesocosm study | Tree species | Young/Mature trees | $CH_4$ emissions measured from |
|---|---|---|---|---|---|
| Rusch & Rennenberg, 1998 | Germany | Mesocosm | *Alnus glutinosa* | Young | Stem surface |
| Vann & Megonigal, 2003 | US | Mesocosm | *Taxodium distichum* | Young | Above ground portion of the entire plant |
| McBain *et al.*, 2004 | Canada | Mesocosm/ partially controlled in situ measurements | *Populus deltoides* × *Populus nigra* | Young | Above ground portion of the entire plant |
| Garnet *et al.*, 2005 | US | Mesocosm | *Taxodium distichum* | Young | Above ground portion of the entire plant |
| Terazawa *et al.*, 2007 | Japan | *In situ* | *Fraxinus mandshurica var. japonica* | Mature | Stem surface |
| Gauci *et al.*, 2010 | UK | *In situ* | *Alnus glutinosa* | Mature | Stem surface |
| Rice *et al.*, 2010 | US | Mesocosm | *Fraxinus latifolia, Populus trichocarpa, Salix fluviatilis* | Young | Entire tree |
| Machacova *et al.*, 2013 | Germany | Mesocosm | *Alnus glutinosa* | Young | Stem surface |

Rice *et al.* (2010) estimated for the first time the source strength of tree-mediated $CH_4$ emissions to be as high as 80 Tg $a^{-1}$, equivalent to 10% of the global $CH_4$ budget and *c.* 30% of the wetland source strength. They used Leaf Area Index (LAI) and fluxes obtained from pot-scale studies to estimate its global importance. They also made the first attempt to measure $CH_4$ egress from the leaves of wetland-adapted trees (*Fraxinus latifolia*, *Populus trichocarpa* and *Salix fluviatilis*), although were unsuccessful in estimating fluxes due to non-linear increase in $CH_4$ concentrations within the tedlar bag leaf-enclosures. Methane released from all three tree species were enriched in carbon isotopic composition ($\delta^{13}C$) and were similar to $\delta^{13}C$ of $CH_4$ produced in C3 plants by aerobic $CH_4$ production mechanisms (Keppler *et al.,* 2006), therefore posing difficulties to distinguish between the aerobic and anaerobic mechanisms of $CH_4$ production based on the $\delta^{13}C$ of emitted $CH_4$ alone (Rice *et al.,* 2010).

A common observation across all these studies was the release of $CH_4$ from wetland trees adapted to flooding with the notable exception being McBain *et al.* (2004) who reported $N_2O$ emissions but not $CH_4$ emissions from hybrid poplar seedlings (*Populus deltoides* × *Populus nigra*), even though morphological adaptations to flooding was evident. The reasons for the absence of $CH_4$ emissions were unclear, but poor solubility of $CH_4$ in soil solution around the tree roots, insufficient development of inter-connected pore spaces in stems, roots failing to aid $CH_4$ diffusion and extensive $CH_4$ oxidation in the soil were suggested by McBain *et al.* (2004) as possible explanations.

### 1.5. Adaptations to flooding and $CH_4$ transport

It is well established that aerenchyma in herbaceous plants and pneumatophores in mangroves mediate gas transport between soil and atmosphere (Skelton & Allaway, 1996;

Purvaja *et al.,* 2004; Kreuzwieser *et al.,* 2008). These adaptations occur in response to soil flooding. Flooding restricts $O_2$ availability in soil, inhibits root formation, branching, growth of existing roots and mycorrhizae, leading to the decay of the root system (Kozlowski, 1997; de Simone *et al.,* 2002, 2003). Furthermore, flooding causes impeded physiological functioning and poses a multiplicity of constraints, including decrease in photosynthesis, adversely affecting plant developmental stages, reduction in absorption of macronutrients due to impeded functioning of root system and in some cases, plant mortality (Megonigal & Day, 1992; Kozlowski, 1997).

In order to overcome these issues, inundated trees adapt through morphological changes, including development of aerenchymatous tissues, adventitious roots and lenticels (Kozlowski, 1997). Wetland-adapted trees display stem thickening due to the growth of bark tissues accompanied by an increase in the proportion of aerenchyma in vascular tissues and modifications in the lower stem and roots to facilitate gas transport to the roots. Such changes are well characterised for herbaceous plants (e.g. Conrad, 1989; Whiting & Chanton, 1992; Bartlett & Harris, 1993; Brix *et al.,* 1992, 1996, 2001; Segers, 1998; Grünfeld & Brix, 1999), flood tolerant angiosperms and gymnosperms in temperate zones (Kozlowski, 1997 and references within) and tropical zones (Parolin, 2001; De Simone *et al.,* 2002; Waldhoff & Parolin, 2010). Morphological adaptations not only enable trees to overcome hypoxia and survive and thrive in wetland ecosystems but also aid gas movement, transporting $O_2$ to the roots from the atmosphere and soil-produced gases in the opposite direction, from the roots to the atmosphere, thus acting as a mechanism to transport soil-produced $CH_4$.

While there is convincing evidence of the connection between morphological adaptation and gas transport, $N_2O$ and $CH_4$ transport also has been reported in an upland tree (*Fagus sylvatica*) grown in aerobic soil that lacks morphological adaptations (Pihlatie *et al.*, 2005;

Machacova *et al.*, 2013). Although, *Fagus sylvatica* are well known for their shallow root system, these observations are not surprising, in most ecosystems, as tree roots grow to depths greater than 1 m, sometimes as deep as 4 m, and are known to have high methanogenic activity initiated through crypto-ephemeral waterlogging (Teh *et al.,* 2005) or elevated dissolved $CH_4$ concentrations in groundwater (Jackson *et al.,* 1999; Teskey & McGuire, 2002). Tree-mediated $CH_4$ emissions from non-wetland environments may be important in forests experiencing temporary or periodic flooding, but does not fall within the scope of this research.

### 1.6. Mechanisms of gas transport

Little is known about tree-mediated $CH_4$ transport mechanisms. Studies of tree-mediated $CH_4$ emissions have provided only a few insights. For example, Terazawa *et al.* (2007) observed $CH_4$ emissions from trees during the leafless season and suggested $CH_4$ transport occurs through internal air spaces in tree bodies. They also observed higher $CH_4$ emissions at lower stem height. Their findings are in agreement with Rusch & Rennenberg (1998) who also reported an apparent decrease in stem-$CH_4$ emission with increasing height and a linear relation between $CH_4$ emitted to the atmosphere and dissolved $CH_4$ in soil. Rusch & Rennenberg (1998) therefore concluded that $CH_4$ transport in trees is mainly driven by diffusion and activated when soil $CH_4$ concentrations exceed atmosphere concentrations, creating a concentration gradient sufficient to transport $CH_4$ from soil to the atmosphere. Garnet *et al.* (2005) present further evidence in favour of $CH_4$ transport via diffusive gas transport, arguing that a lack of mid-morning $CH_4$ emission peak and the non-hysteretic $CH_4$ emission response curve favour the hypothesis of diffusion driven $CH_4$ transport over pressurised gas transport.

Another transport pathway, which is often discussed but seldom studied, is $CH_4$ transport through transpiration. For an actively transpiring tree, $CH_4$ may be transported by the transpiration stream from the roots to the leaves and emitted to the atmosphere through the stomata (Chang *et al.,* 1998), or stem surfaces (diffusing laterally and radially through intercellular spaces of the aerenchyma system), similar to $CH_4$ transport observed and documented for a variety of wetland plant species (e.g., Brix *et al.,* 2001; Conrad, 1989; Whiting & Chanton, 1992; Grünfeld & Brix, 1999; Machacova *et al.*, 2013). Given that trees support a high evapotranspiration flux, trees may provide preferential pathways to release soil produced $CH_4$. However, $CH_4$ transport potential via the transpiration stream may be orders of magnitude lower than the transport via other mechanisms as $CH_4$ is relatively insoluble in water (Conrad, 2009). Yet, preliminary studies conducted by Rice *et al.* (2010) and Terazawa *et al.* (2007) report very high dissolved pore-water $CH_4$ concentrations and therefore the possibilities of $CH_4$ emission through a transpiration pathway cannot be ruled out.

Based on these studies, mechanisms of tree-mediated $CH_4$ transport may be divided into two categories: emission driven by diffusion gradient and transpiration stream (Gauci *et al.,* 2010). However, the current knowledge of the mechanisms responsible for herbaceous plant-mediated $CH_4$ emissions, $O_2$ transport in wetland trees and $CO_2$ transport in trees is extensive and may offer valuable insight into the likely tree-mediated $CH_4$ emissions transport mechanisms, all of which are briefly discussed below.

### *1.6.1. Plant-mediated $CH_4$ emissions*

Two gas transport mechanisms, transport via molecular diffusion and convective through flow are proposed for emergent wetland plants. Wetland plant species such as *Carex gracilis*, *Oryza sativa and Peltandra virginica* are documented to employ molecular diffusion driven $CH_4$ transport (e.g., Seiler *et al.,* 1984; Chanton *et al.,* 1993). While, other

wetland species such as *Eleocharis sphacelata, Phragmites australis* and *Typha spp* employ convective through flow, mostly in addition to diffusion driven $CH_4$ transport, in which $CH_4$ flows from a region of high pressure to lower pressure (e.g., Chanton & Whiting, 1996; Whiting & Chanton, 1996; Käki & Kankaala, 2001). The necessary pressure differential may be accomplished by one or more mechanisms, including humidity (humidity-induced convection), thermal (thermo-osmosis), wind speed (venture-induced convection) differential across plant lacunal tissue (Grosse *et al.,* 1991; Schütz *et al.,* 1991; Armstrong *et al.,* 1992; Brix *et al.,* 1992) and stomatal conductance (Morrissey *et al.,* 1993; Kim *et al.,* 1998). In general, rates of $CH_4$ transport are higher when plants employ convective gas transport and/or both convective and molecular diffusion transport than those that solely employ molecular diffusion (Chanton *et al.,* 1993; Whiting & Chanton, 1996).

*1.6.2. Oxygen transport in wetland trees*

Several mechanisms for $O_2$ transport in the aerenchyma have been discussed and may be relevant to tree-mediated $CH_4$ transport. These mechanisms are mostly similar to plant-mediated $CH_4$ emission mechanisms, except the gas transport is in the opposite direction. These mechanisms include: i) $O_2$ transport by diffusion following Fick's law (e.g., Armstrong, 1971; Brix, 1988); ii) photosynthesis induced $O_2$ diffusion (e.g., Grosse, 1996; Dittert *et al.,* 2006); and iii) transport through pressure gradient: humidity induced diffusion and thermo-osmotic diffusion (e.g., Armstrong *et al.,* 1992, 1996). Respiratory consumption in the rhizosphere and $O_2$ release during photosynthesis create an $O_2$ concentration gradient diffusing $O_2$ downwards in the aerenchyma along the concentration gradient, enabling $O_2$ transport (e.g., Armstrong, 1971; Brix, 1988; Schütz *et al.,* 1991; Whalen, 2005; Dittert *et al.,* 2006). While, the concentration gradient caused by the difference between the outside (lower humidity) and inside (higher humidity) of the plants

(Dacey, 1981; Brix, 1988) drives humidity induced diffusive $O_2$ transport, the temperature difference between the stem and ambient air, typically observed in tropical forests, drives thermo-osmotic $O_2$ transport (Armstrong *et al.,* 1992; Brix *et al.,* 1992; Grosse, 1996; Dittert *et al.,* 2006).

### *1.6.3. Carbon dioxide transport in trees*

The internal transport of dissolved $CO_2$ from below ground largely derived from root-respiration, assimilation and subsequent release in trees has been the focus of several recent studies (e.g., Teskey & McGuire, 2002, 2005; McGuire & Teskey, 2004; Aubrey & Teskey, 2009; Bloemen *et al.,* 2013). These studies report root-respired $CO_2$ to be transported internally upwards in the tree and diffused to the atmosphere via the transpiration stream - a mechanism already proposed to drive tree-mediated $CH_4$ emissions. Furthermore, the concentrations of $CO_2$ in the xylem exceeding many times that of the atmosphere is responsible for such transport (Teskey & McGuire, 2005; McGuire *et al.,* 2007).

## 1.7. Controls on $CH_4$ emissions from trees

While studies on tree-mediated $CH_4$ emissions are limited, it is instructive to consider several environmental factors that are known to control plant-mediated $CH_4$ transport, $CH_4$ production and oxidation. These factors are proposed to be important for tree-mediated $CH_4$ emissions and three key factors, wetland vegetation, water-table depth and temperature, are discussed below.

### *1.7.1. Wetland vegetation*

Wetland vegetation can enhance and attenuate methanogenic and methanotrophic activities and are therefore proposed to affect tree-mediated $CH_4$ emissions. The transport of $O_2$ via wetland vegetation enriches $O_2$ in the rhizosphere, thereby suppressing methanogenesis and stimulating below-ground $CH_4$ oxidation (Chanton & Whiting, 1996; Christensen *et al.,* 2000), nitrogen fixation (Reay *et al.,* 2001, 2005) and oxidation of other electron acceptors (Sutton-Grier & Megonigal, 2011). Alternatively, wetland vegetation mediates $CH_4$ emission not only by offering a preferential pathway to release $CH_4$ from the point of production to the atmosphere (e.g., Whiting & Chanton, 1992, 1993; Greenup *et al.,* 2000; Ström *et al.,* 2003), but also by supplying additional carbon source (e.g., allocation of recently fixed carbon to the roots) which stimulates methanogenesis (Updegraft *et al.,* 1995; Chanton, 1995; Joabsson *et al.,* 1999; Greenup *et al.,* 2000; Ström *et al.,* 2003).

Different species influence $CH_4$ emissions from wetlands differently. For example, the decrease in $CH_4$ emissions after wetland-vegetation removal has been demonstrated in numerous studies and the emissions response is generally consistent between studies; however, there also have been reports of $CH_4$ emission reduction in the presence of wetland vegetation, and in both cases the magnitude varied within and between studies (Schimel, 1995; Greenup *et al.,* 2000; Dinsmore *et al.,* 2009; van Winden *et al.,* 2012). Various authors also report contrasting relationships between plant biomass, net ecosystem exchange and $CH_4$ emissions (e.g., Whiting & Chanton, 1993; Waddington *et al.,* 1996; Joabsson & Christensen, 2001; Ström *et al.,* 2005; von Fischer *et al.,* 2010). These differences are attributed to species-specific differences in assimilation (Whiting & Chanton, 1993; Ström *et al.,* 2005; Sutton-Grier & Megonigal, 2011), carbon allocation (Shaver & Kummerow, 1992; Ström *et al.,* 2005, 2012), vegetation height and biomass (Joabsson *et al.,* 1999, Joabsson & Christensen, 2001; Ström *et al.,* 2003; Kutzbach *et al.,*

2004; von Fischer *et al.,* 2010), morphological adaptation (Kozlowski, 1997; Segers, 1998), root depth, architecture and morphology (Frenzel & Rudolph, 1998; von Fischer *et al.,* 2010; Sutton-Grier & Megonigal, 2011), $CH_4$ oxidation capacity (Frenzel & Rudolph, 1998; King *et al.,* 1998; Frenzel, 2000; Ström *et al.,* 2005) and plant-root-microbial consortium (Moore *et al.,* 2002; Bubier *et al.,* 2003; Christensen *et al.,* 2004; Johansson *et al.,* 2006; Sutton-Grier & Megonigal, 2011).

*1.7.2. Soil and air temperature*

Temperature is well-known to affect $CH_4$ emissions from wetland vegetation and is also proposed to influence tree-mediated $CH_4$ emissions. Strong positive relationships (exponential or linear) between air and soil temperature and $CH_4$ emission has been well characterised in a wide range of ecosystems (e.g., Dise *et al.,* 1993; Shannon & White, 1994; van Bodegom & Stams, 1999; Dinsmore *et al.,* 2009), with temperature variations accounting for up to 84% of the observed variations in $CH_4$ emissions (Christensen *et al.,* 2003).

According to several classic (Crill *et al.,* 1988; Dise *et al.,* 1993; MacDonald *et al.,* 1998) and recent (van Bodegom & Stams, 1999; Gauci *et al.,* 2002; Dinsmore *et al.,* 2009; van Winden *et al.,* 2012) studies, increasing temperature can affect $CH_4$ emissions via direct temperature effects on metabolic rates of methanogens and methanotrophs, and indirectly in several ways, e.g., through changes in plant physiological and net ecosystem productivity (NEP), and shift in plant communities, density and composition, which are known to stimulate substrate availability and microbial activity, resulting in higher $CH_4$ production (Nadelhoffer *et al.,* 1991; Whiting & Chanton, 1992; King *et al.,* 1998; Grünfeld & Brix, 1999; van Winden *et al.,* 2012). Such strong temperature effects are also responsible for the annual $CH_4$ variations observed in mid-high latitudes (Williams & Crawford, 1984; Crill *et al.,* 1988; Gauci *et al.,* 2004), i.e., higher $CH_4$ fluxes in summer

due to higher temperature and lower fluxes in winter. However, such temperature-dependence is absent or attenuated in tropical wetlands, where water-table fluctuations have the greatest influence on annual variations in $CH_4$ flux (Jauhiainen *et al.,* 2005).

*1.7.3. Water-table depths*

Changes in water-table depths may directly and indirectly affect herbaceous plant- and tree-mediated $CH_4$ emissions. Water-table depths affect the degree of anaerobic conditions and the depth of aerobic layer, consequently the ratio of $CH_4$ production and oxidation (Christensen *et al.,* 2001). As a result, water-table depths are regarded as the primary controlling factor on $CH_4$ production (e.g., Dise *et al.,* 1993; Macdonald *et al.,* 1998; Turetsky *et al.,* 2002; McNamara *et al.,* 2006). Therefore, the role of water-table depths on $CH_4$ emissions, particularly on plant-mediated $CH_4$ emissions, has been studied extensively in varied environment both in temperate and tropical ecosystems (e.g., Dise *et al.,* 1993; MacDonald *et al.,* 1998; Grünfeld & Brix, 1999; Turetsky *et al.,* 2002, 2008; Jauhiainen *et al.,* 2005; Dinsmore *et al.,* 2009).

Water-table depths indirectly affect herbaceous plant- and tree- mediated $CH_4$ emissions due to its ability to alter assimilation, growth and root distribution, and consequently affect methanogenesis and $CH_4$ transport (e.g., Vann & Megonigal, 2003; Dinsmore *et al.,* 2009; Ström *et al.,* 2012). Blodau *et al.* (2004) demonstrated a 24% and 42% drop in assimilation rate in two Canadian peatlands, when the water-table was lowered by 30 cm, similar to the 21-44% reduction observed by Dinsmore *et al.* (2009). In contrast, other studies have shown that drier soil conditions increase below-ground productivity of emergent plants (Weltzin *et al.,* 2000), thereby stimulating $CH_4$ emissions through increased availability of labile carbon substrates in soil via root exudation and by increasing $CH_4$ transport to the surface due to shifting rooting zones (Strack *et al.,* 2006). These studies indicate the complexity in plant-mediated $CH_4$ emission response to water-table depth variations.

## 1.8. Knowledge Gap

A few studies have so far demonstrated stem-$CH_4$ emissions (e.g., Rusch & Rennenberg, 1998; Terazawa *et al.,* 2007; Gauci *et al.,* 2010), but the underlying mechanisms responsible for tree-mediated $CH_4$ emissions still remain unknown. Methane emissions from mature and young trees in temperate regions and certain controlling factors have been documented (e.g., Rusch & Rennenberg, 1998; Vann & Megonigal, 2003; Garnet *et al.,* 2005) but how these emissions vary over longer time scales and their relevance to tropical forested ecosystems is still uncertain. Importantly, measuring tree-mediated $CH_4$ emissions and estimating its ecosystem contributions have received very little attention. Given that *c.* 60% of all wetlands are forested (Mathews & Fung, 1987) and that many tropical forests are either permanently or seasonally flooded, tree-mediated $CH_4$ emissions from wetland-adapted trees represent an important research area that has implications for understanding and constraining the global $CH_4$ budget.

## 1.9. Research aims and objectives

In order to elucidate the capacity of wetland-adapted trees to transport and emit soil-produced $CH_4$ to the atmosphere, this study aims to understand the role of tree-mediated $CH_4$ emission pathway relative to other well-known $CH_4$ emission pathways in a temperate and tropical forested wetland and to assess its ecosystem contribution. The study also aims to characterise the temporal variability and controls on tree-mediated $CH_4$ emission.

*1.9.1. Research objectives*

The specific objectives of this study are to:

**Obj.1.** Assess the presence or absence of tree-mediated $CH_4$ emissions from wetland-adapted trees (both tropical and temperate).

**Obj.2.** Assess the spatial and temporal variability of $CH_4$ emissions along the height of the tree and between different trees species.

**Obj.3.** Investigate the mechanisms responsible for transport and release of $CH_4$ by wetland trees.

**Obj.4.** Identify and characterise key environmental variables affecting tree-mediated $CH_4$ emissions.

**Obj.5.** Evaluate the role of trees in forested wetland $CH_4$ emissions and establish an ecosystem-scale $CH_4$ budget by quantifying $CH_4$ emissions from wetland-adapted trees and soil surface components.

## 1.10. Structure of the thesis

This thesis is organised in six chapters. **Chapter one** presents the background for key aspects of research carried out in the thesis, highlighting the knowledge gaps and presenting the aims and objectives of the research. **Chapter two** describes the field sites used during the investigation and the general methods employed. Additionally, specific methods and field-site descriptions are included within each chapter.

**Chapters three, four and five** are written in paper format, and include a brief introduction, specific methods, results and discussions associated with each of the aspects

investigated (i.e., controls, ecosystem contributions of temperate and tropical wetland trees). While **Chapter three** identifies the controls on tree-mediated $CH_4$ emissions using a partially controlled mesocosm experiment, **Chapter four** tackles the principal aim of the thesis by quantifying the ecosystem contribution of tree-mediated $CH_4$ emission and its spatial and temporal variability in temperate forested wetland. In addition it also presents further controls on tree-mediated $CH_4$ emissions *in situ*. **Chapter five** focuses on extending these findings to the tropical forested wetland by quantifying tree-mediated $CH_4$ emission and ecosystem contributions in comparison with other $CH_4$ emission pathways. Finally, **Chapter six** acts as a short overall discussion combining the findings of the three previous data chapters, underlining implications, placing the findings in a global context and summarising the major findings of the study, with recommendations for further work.

# CHAPTER TWO

# Methodology

## 2.1. Introduction

This chapter describes the field sites monitored, and the generic methods used throughout the investigation that are referred back to in subsequent chapters, such as use of static chambers, sample collection, flux calculations and statistical analyses. The more specific methods that are tailored to answer particular research objectives are discussed within each of the experimental chapters.

## 2.2. Site description

The investigation was carried out at two different scales: a partially controlled mesocosm experiment and *in situ* field monitoring, with field investigations carried out in a temperate forested wetland (intensive study spread over a year) and a tropical forested wetland (short pilot study).

*2.2.1. Temperate forested wetland*

Methane emissions were measured in a temperate spring-fed forested wetland (*c.* 59 ha), a valley mire system of alkaline fen and acidic springs, mosaic of fens, meadows and wet woodlands, located in Flitwick, Bedfordshire, UK (52° 0' N, 0° 28' W) about 45 miles north of London (Fig. 2.1). The wetland overlies *c.* 10 m of greensand (Woburn sands), overlain by Gault clay and the surface soils comprise of gravels, alluvial deposits and peat. The peat was formed as a result of ground water upwelling from the underlying greensand aquifer into a river valley leading to the accumulation of organic matter since the last Ice Age. As a result, it is one of the most important wetlands in south-east England and is a Site of Special Scientific Interest (SSSI) located at grid reference TL 045350, owned and managed by the Wildlife Trust for Bedfordshire, Cambridgeshire and Northamptonshire.

Figure 2.1: Map of the UK with black dot displaying the location of the study site, where $CH_4$ emissions were measured for a year.

The wetland contains a rich assemblage of vascular lower plants and carr woodland and is renowned for both its flora and invertebrate fauna, as well as being of national importance for mosses and fungi. The site is dominated by the wetland-adapted tree species, *Alnus glutinosa* (L.) Gaertner and *Betula pubescens* (Ehrh.), with *Alnus glutinosa* dominating some parts of the wetland. The understorey of the forest is covered by large stands of *Phragmites australis, Typha latifolia, Holcus lanatus, Lythrum salicaria, Scrophularia auriculata, Alisma plantago-aquatica, Potamogeton spp., Carex spp.* and *Sphagnum spp.* The spring fed water and river Flit (susceptible to occasional flooding) that flows through the peatland drives local hydrology and typically maintains the water-table near the surface year round, including within hummocks.

Figure 2.2: Temperate forested wetland study plot showing stands of *Betula pubescens*.

The climate is temperate with average summer and winter temperatures of 15.5 °C and 3.9 °C, respectively, and a 10-year precipitation average of 647 mm $a^{-1}$ (576 mm $a^{-1}$ during the study period; Environment Agency rain gauge, Toddington, 5 km SW of the study site). The observation period was an atypical year with a longer growing season, a late autumn and a short and relatively warm winter.

*2.2.1.1. Study plot*

A 20 × 30 m plot was selected on the southeast side of the peatland on the basis of its accessibility (Fig. 2.2). The study plot contained 10-20 m tall *Alnus glutinosa* and *Betula pubescens* trees. In addition, *Phragmites australis* and *Carex spp.* were abundant and were predominantly found on hummocks. The plot was characterised by mapping the location of both the tree species along with the distribution of hollows and hummocks (vegetated and non-vegetated). The percentage distribution of the hummock and hollow was estimated to be 65% and 35% respectively, and stayed relatively constant throughout the observation period due to the upwelling hydrology. The stem diameter of all trees (≥ 7 cm) was measured at 1.3 m height (diameter at breast height, DBH) and the basal diameter was estimated by measuring the stem diameter at 10 cm above the soil surface. These trees were categorised as mature trees. The stem diameter, basal diameter and distribution of trees ≤ 7 cm were also measured and these were categorised as young trees. The density of all trees (both categories) within the plot also was calculated. Approximately 92% of the trees measured had a DBH ≤ 20 cm (Fig. 2.3).

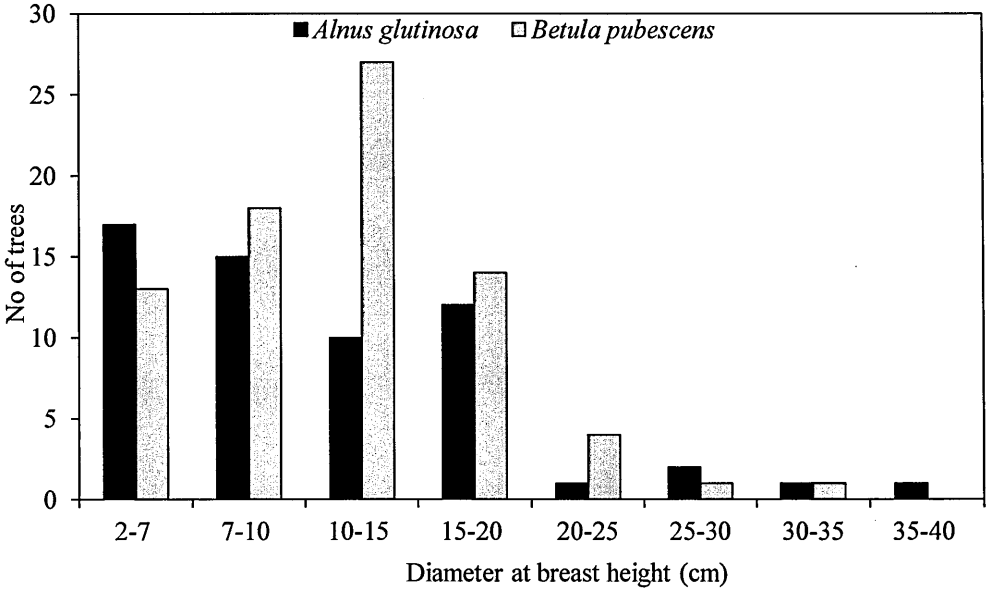


Figure 2.3: The range of tree diameters measured at 1.3 m stem height within the 20 × 30 m study plot.

The phenology within the study plot was carefully documented throughout the observation period. Live under-storey vegetation started to appear in late April, 2011, growing to full height (1.2 m) in May and approached dormancy by November. Fully expanded tree leaves were observed at the beginning of May 2011 on both the tree species, while autumnal leaf senescence was observed in November followed by a short vegetative dormancy between December and February. Early bud burst, under-storey vegetation growth (by March 2012) and fully expanded leaves were observed by the end of April 2012.

### *2.2.2. Tropical forested wetland*

Methane fluxes from tree and soil surfaces were measured during a two-week period in March, 2011 during the wet season in a tropical forested peatland situated in the upper Sebangau River catchment in Borneo, Indonesia (2° 20' S, 113° 55' E). The relatively undisturbed forested peatland is located *c.* 20 km southeast of Palanka Raya city in Central Kalimantan (Fig. 2.4) and has been described previously by Page *et al.* (1999).

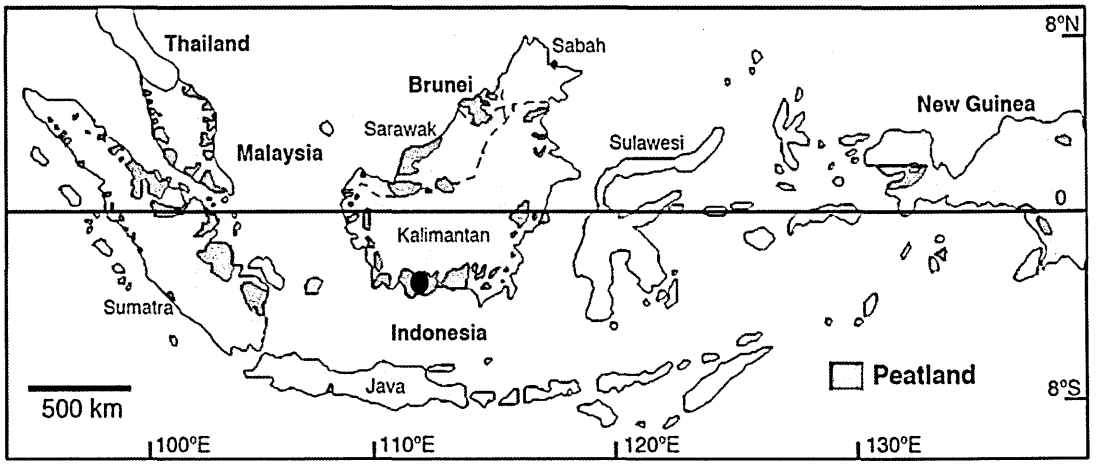


Figure 2.4: Map of the distribution of peatlands in Southeast Asia with the black dot showing the location of the study site, where $CH_4$ emissions were measured for 15 days.

Based on tree species composition and forest structure, three principal peat swamp forest sub-types have been described from this site (Shepherd *et al.*, 1997; Page *et al.*, 1999). Mixed swamp forest dominates the zone beyond the limit of river flooding on the margins of the peat dome, up to a distance of 6 km from the river on peat up to 6 m thick. The dominant tree species in these forests are *Gonystylus bancanus*, *Shorea spp.*, *Cratoxylon glaucum* and *Dactylocladus stenostachys*. Mixed swamp forest continues into low pole forest which extends for a further *c.* 7 km and the principal species include *Combretocarpus rotundatus* and *Calophyllum spp.* Due to the higher light levels penetrating the canopy, and the permanently high water-table in this forest zone, there is a dense understorey of *Pandanus* and *Freycinetia spp.* The summit of the watershed is occupied by forest with a much taller canopy, known as tall interior forest, where the peat thickness is 10-13 m. Tree species of the genera *Agathis*, *Dactylocladus*, *Gonystylus*, *Koompassia*, *Palaquium* and *Shorea* are abundant in these forests (Shepherd *et al.*, 1997; Page *et al.*, 1999).

The humid tropical climate is characterised by uniform temperature, high humidity and high rainfall intensity (*c.* 2800 mm $a^{-1}$). Annual rainfall pattern is determined by two main monsoon systems: a southeast dry monsoon and a northeast wet monsoon. Typically, the wet season lasts from October to May and the dry season lasts from June to September. As a result, the water-table in the forests is above the soil surface during the wet season (*c.* 9 months), decreasing to 40 cm below the peat surface during the dry season (*c.* 3 months). During the study period, the water-table depths were 4.7 ± 1.2 cm above the soil surface and 16 ± 3.5 cm below the soil surface in hollows and hummocks, respectively. The mean air and soil temperatures during the study period were 26.8 ± 2.2 °C and 24 ± 1.0 °C, respectively. Temperatures in the region are usually relatively stable throughout the year, displaying negligible temporal variation (Jauhiainen *et al.,* 2005).

*2.2.2.1. Study plot*

Two study plots (20 × 20 m) *c.* 1 km apart, were established within mixed peat swamp forest (Fig. 2.5) on the basis of its accessibility, a forest type that extends up to 6 km from the margin of the peat dome into the interior, located beyond the zone of river flooding, having a peat thickness ranging from 2-6 m.

Figure 2.5: Tropical forested wetland study plot showing stands of *Cratoxylum arborescens, Shorea balangeran* and *Diospyros bantamensis.*

The locations and distribution of trees, hollows and hummocks were mapped in each plot. The average area ratio of hollows to hummocks was 50:50 in the plots (56.4 : 43.6 in Plot 1 and 43.5 : 56.5 in Plot 2). Tree species in each plot were identified and every tree having a diameter $\geq$ 7 cm at 1.3 m height above soil surface (DBH) was measured for basal diameter and stand density. Approximately 87% of the trees measured had a DBH $\leq$ 20 cm (Fig. 2.6), similar to the DBH distribution reported for SE Asian tropical peat forests (Page *et al.,* 1999 and references within) and some Amazonian forests (Macía, 2011). Stem diameter also was measured at 10 cm intervals between 20 and 130 cm above the soil surface for each of the eight tree species identified for $CH_4$ measurements. These eight dominant tree species within the two plots were: *Mesua* sp. 1, *Xylopia fusca* Maingay ex Hook. f. & Thomson, *Shorea balangeran* (Korth.) Burck, *Diospyros bantamensis* Koord.

& Valeton ex Bakh., *Tristaniopsis* sp. 2, *Litsea elliptica* Blume, *Elaeocarpus mastersii* King and *Cratoxylum arborescens* (Vahl) Blume.

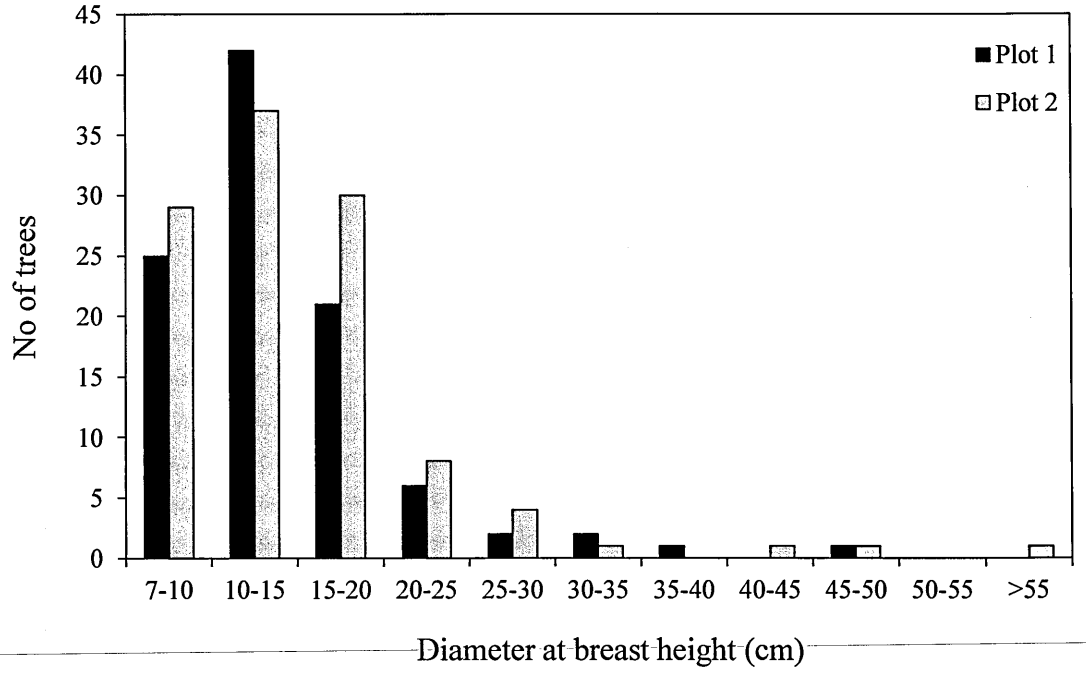


Figure 2.6: The range of tree diameters measured at 1.3 m stem height (DBH ≥ 7 cm) within the two 20 × 20 m plots.

*2.2.3. Mesocosm experiment*

The mesocosms consisted of a cylindrical container constructed of durable polyvinyl chloride (diameter 36 cm, height 55 cm). A 5 cm drainage layer was formed at the bottom of each pot using 10 mm gravel. This material was overlaid with a 45 cm thick mixture of 95% commercial sphagnum peat and 5% top soil, on a volume basis (MANRO South, Cambridgeshire, UK). The layers were separated using a woven polyester fabric, which impeded root growth into the drainage layer and prevented the overlying peat soil from blocking the drainage layer. 200 g of peat soil from Flitwick Moor, temperate forested wetland (the study plot) was also added to each mesocosm. Three-year old *Alnus glutinosa* saplings purchased from Hedge Nursery, Hereford, UK were planted in the peat mixture in

2010. The mesocosms were divided into two treatments based upon water-table position: one at the soil surface (HW) and the other 25 cm below the soil surface (LW). The 24 replicates of each treatment were arranged randomly outdoors, in a non-shaded area of the Open University campus in Milton Keynes, UK (Fig. 2.7).

Figure 2.7: The mesocosm setup consisting of 4-yr old *Alnus glutinosa* planted in organic soil mixture and maintained at two water-table depths (at the surface and 25 cm below the surface).

Water-table levels were maintained at the desired depth in the mesocosms using the method reported by Araya *et al.* (2010), which involved controlling water levels using a reservoir tank and two float chambers fitted with ball-valves. Ball-valves regulated water flow from the reservoir tank to the 0.1 $m^3$ float chambers and subsequently into the mesocosms thereby automatically regulating water-table levels to compensate for evaporative losses. The float chambers were connected by branching hosepipes (diameter 1.25 cm) to the bottom of the individual mesocosms. Water-levels in the control chambers

and the mesocosms were set using Total Station® surveying equipment (T705, Leica Geosystems®, St Gallen, Switzerland).

Mains water supplied to the reservoir tank (1.5 $m^3$) was kept anoxic through contact with dried sugar beet shreds held within a porous sack and renewed monthly at a rate of 5 kg $m^{-3}$ of water (Araya *et al.,* 2010). The dried sugar beet deoxygenated the water but also introduced acetate, a known methanogenic substrate, to the mesocosms. Analysis of water samples from the reservoir showed a 92% reduction in dissolved $O_2$ (from 0.25 mmol at the inlet to 0.02 mmol at the outlet) and an increase in acetate concentration from below detection limit at the inlet to 0.18 mmol at the outlet. The latter concentration of dissolved acetate is comparable to quantities that commonly occur in peatland soils (e.g., Shannon & White, 1996) and should have enhanced production of $CH_4$ in the mesocosms, facilitating assessment of gas transport mechanisms and pathways in *Alnus glutinosa*. The acetate concentrations in water were measured fortnightly at the outlet of the reservoir tank, inlet and outlet of the float chambers to ensure that all the mesocosms (n = 48; LW and HW mesocosms) received the same concentrations of acetate.

*Alnus glutinosa* was chosen for the mesocosm experiment because of their well-known ability to adapt to wet soil and mediate gas transport (e.g., Rusch & Rennenberg, 1998; Gauci *et al.,* 2010). The mesocosm experiment also complemented the *in situ* $CH_4$ measurements from mature *Alnus glutinosa* at Flitwick Moor temperate forested wetland.

### 2.3. Static chambers

Closed self-contained, custom designed static chambers were used to measure $CH_4$ emissions from the soil and stem surfaces *in situ* and were analysed using a modified cavity ring down laser spectroscopy (CRDLS; explained in section 2.5). A static chamber method was chosen to measure $CH_4$ emissions owning to its low cost and maintenance,

non-labour intensive, portability and ability to make measurements over a wide range of wetland topography. On the other hand, dynamic chambers that circulate the air over the stem and soil surface using an inlet and outlet connected to the CRDLS were used to measure $CH_4$ emissions from mesocosms (explained in section 2.5). However, due to the difficulty in carrying the CRDLS in wet forests, emissions were monitored by extracting a series of syringe gas samples from the static chambers and analysed using the modified CRDLS. A vent tube (Hutchinson & Livingston, 2001) was incorporated in all chambers to eliminate temperature and pressure changes during sampling. The static and dynamic chambers were tested to ensure that the empty chambers showed neither a decrease in $CH_4$, associated with adsorption of $CH_4$ molecules onto the surface of chamber materials nor an increase in $CH_4$ caused by photo-degradation of acrylic or plastic. The recorded changes in $CH_4$ concentration inside the chambers over time were therefore not a result of any artificial sources.

*2.3.1. Stem static chambers used in situ*

Static chambers used to measure $CH_4$ fluxes from tree stems were constructed from a design described by Rusch & Rennenberg (1998) and Gauci *et al.* (2010) with further modification (Fig. 2.8). The stem-static chamber was constructed from flat Perspex® (Perspex Distribution Tamworth, Tamworth, UK) sheets (30 × 30 × 30 cm) assembled into a cube, which was then cut into two halves and held together using hinges and spring clips. Each cubic chamber had a 20 cm diameter central opening to enclose the tree stem. A clear Perspex cylinder (20 cm diameter × 5 cm height) was attached to the central opening on either side of the chamber, which held a gas-impermeable foam strip (7 cm wide) against the tree stem, creating a gas-tight seal. A transparent sheet of gas-impermeable fluorinated ethylene propylene film (FEP; Adtech Ltd, Gloucestershire, UK) was attached to the

outside of the Perspex cylinder, the foam strips and tree stem to further strengthen the gas-tight seal. Each chamber also contained a gas sampling port and pressure regulator. Pressure, temperature and humidity inside the stem chamber were continuously logged (TR-73U thermo recorder; T & D Corporations, Nagano, Japan) during sample collection.

Figure 2.8: Static chambers used to measure tree stem-$CH_4$ emissions.

Methane emissions from the stems of two wetland tree species (*Alnus glutinosa* and *Betula pubescens*) in the temperate forested wetland (with a stem diameter of 7.5-19.5 cm) and eight wetland tree species (*Mesua* sp. 1, *Xylopia fusca*, *Shorea balangeran*, *Diospyros bantamensis*, *Tristaniopsis* sp. 2, *Litsea elliptica*, *Elaeocarpus mastersii* and *Cratoxylum arborescens*) in the tropical forested wetland (with a stem diameter of 7.5-19.5 cm), were measured at three heights: 20-50 cm, 60-90 cm and 100-130 cm above the soil surface. However, in the temperate forested wetland, in order to investigate the emissions along the length of the tree, $CH_4$ emissions were measured at an additional stem height (140-170 cm) for two trees of each species on each sampling occasion. During each measurement, $CH_4$ emissions were simultaneously measured from two trees (different tree species) at three stem height positions within a 2 m radius. This allowed comparison of both $CH_4$ emissions between the two tree species at a specific stem height and emissions at three stem heights for each tree and between tree species.

*2.3.2. Soil static chambers – temperate forested wetland*

Static chambers, used to measure $CH_4$ emissions from the hollows and hummocks (non-vegetated; six each) were constructed using polyvinyl chloride (PVC) collars (30 cm diameter × 25 cm height) inserted 5 cm into the soil surface in order to ensure that the chambers are positioned securely and the disturbance during chamber lid deployment is minimised. A transparent lid (30 cm diameter × 0.8 cm thickness) equipped with a pressure regulator and sampling port enclosed the soil collars prior to each gas sampling event (Fig. 2.9). Static chambers used to measure $CH_4$ emissions from the hollows and hummocks (vegetated; four each), were constructed using a circular aluminium wire mesh sandwiched between two sheets of gas impermeable FEP films (36 cm diameter × 140 cm height) inserted 10 cm into the soil surface, and these enclosed both the vegetation and the soil

surface. An acrylic lid (36 cm diameter × 0.8 cm thickness) equipped with a pressure regulator and sampling port enclosed the soil collars. The soil collars were installed two weeks prior to the experiment and were left in place until the end of the experiment. Care was taken while enclosing the soil chambers to minimise disturbance and data that displayed evidence of induced ebullition at t = 0 were rejected (~8% of gas samples analysed).

Figure 2.9: Static chambers used to measure $CH_4$ emissions from hollows and hummocks (non-vegetated).

### *2.3.3. Soil static chambers – tropical forested wetland*

Static chambers used to measure $CH_4$ fluxes from six locations per plot in ponded hollows and hummocks in the tropical forested wetland were based on the design described by Gauci *et al.* (2002). Approximately 30 fluxes were measured from each hollow and hummock per plot (i.e., 120 measurements in total). The static chambers were constructed from PVC pipe and deployed on permanently installed soil collars (35 cm diameter × 30 cm height) inserted 5 cm into the peat surface 2-day before gas sampling. Each chamber had a removable lid equipped with a pressure regulator and sampling port. Static chambers used to measure $CH_4$ fluxes from pneumatophores were constructed from PVC rectangular

collars (40 × 30 × 40 cm) inserted 5 cm into the soil surface and enclosed one to three pneumatophores. During each measurement, a rectangular lid containing a gas sampling port and pressure regulator was placed on the collars.

*2.3.4. Static chambers – mesocosm experiment*

The static chambers used in the mesocosm experiment were equipped with an inlet and outlet port in order to measure $CH_4$ in real time using CRDLS, a feature common across all the chambers described below. Additionally, a small needle hole (0.8 mm) in a resealing membrane (Suba Seal, Sigma-Aldrich, St. Louis, MO, USA) allowed pressure to be controlled in all the chambers.

Static chambers used to measure $CH_4$ flux from the soil surface were based on a design described by Boardman *et al.* (2011) and were constructed from PVC pipe, consisting of a soil collar (8.2 cm diameter x 10 cm height) permanently inserted 4 cm into the soil in each mesocosm and a removable headspace chamber (8.2 cm diameter x 25 cm height) equipped with a pressure regulator and sampling port on the transparent lid (Fig. 2.10). The removable headspace chamber was attached to the soil collar during each deployment.

Figure 2.10: Static chambers used to measure soil $CH_4$ emissions from the mesocosms.

The static chamber used to measure $CH_4$ emissions from the whole-mesocosm (i.e. tree plus soil $CH_4$ emissions; Fig. 2.11) was constructed from reinforced transparent sheets of gas-impermeable FEP film fitted on a cylinder constructed of wire mesh (36 cm diameter x 150 cm height). During sampling, the chamber was covered with a clear Perspex® lid fitted with gas sampling ports and 12V battery-powered fan. The chamber enclosed the entire tree and soil surface.

Figure 2.11: Static chambers used to measure whole-mesocosm $CH_4$ emissions.

Stem surface $CH_4$ emissions were measured at two stem height positions (2-12 cm and 12-22 cm above the soil surface) using a modified version of the method employed by Rusch & Rennenberg (1998). The stem surface chambers were constructed of clear Perspex® cylinders (11 cm diameter x 17 cm height; Fig. 2.12) cut into two halves with a 7 cm hole drilled in the centre to enclose the tree stem. To this opening, at both ends, half a section of the 7 cm diameter Perspex® cylinder (2 cm height) was glued and a gas impermeable neoprene foam strip (0.8 cm thick) was attached to the inside of the cylinder. Both the ends of the cylinder were held together using a custom-made jubilee clip and an air-tight seal between the stem surface and chamber opening was achieved by strapping a gas impermeable neoprene foam strip around the tree stem. A neoprene cord (0.5 cm thick) attached to the cut ends of the cylinders strengthened the air tight seal.

Figure 2.12: Static chamber used to measure stem-$CH_4$ emissions from the mesocosm.

Leaf static chambers were constructed from reinforced transparent sheets of gas-impermeable FEP film fixed on a frame of four adjustable solid aluminium rods attached to

flat Perspex® sheets (6 x 6 cm) fitted with gas sampling ports on one end. The Perspex® sheet attached to the branch was cut into two halves and contained a central opening (2 cm diameter) to enclose the branch. A gas tight seal was achieved by attaching gas impermeable closed-cell foam strips (3 cm wide) onto the branch to which the Perspex® leaf chamber was fitted. The FEP film was permanently fixed to one end of the Perspex® sheet and the other end was attached to the Perspex® sheet using an elastic chord and putty (Terostat IX, Teroson, Henkel, Germany). The elastic chord and putty enabled the chamber to exclude a large portion of the main branch, enclosing only 8-10 leaves during each deployment. Leaf petioles and the branch to which the petioles were attached could not be excluded using this technique.

## 2.4. Pore-water $CH_4$ samplers

### *2.4.1. Temperate forested wetland*

The pore-water $CH_4$ concentrations were measured at five locations within the study plot: two on hummocks and three on hollows, using pore-water equilibrators (Fig. 2.13). Briefly, gas permeable silicon tubing (8 mm in diameter) was wrapped in 5 cm interval slots cut into a PVC column (80 cm long) at 11 depths. The internal volume of the silicon tube was ~17 $cm^3$ for each 5 cm interval. Each end of the silicon tube was fitted with a barbed nylon reduction fitting to which a length of gas impermeable polyurethane tubing (3 mm diameter) was attached and extended to the ground surface. One end of the polyurethane tube was fitted with a three-way gas-tight valve which enabled gas to be sampled from specific depths using gas-tight syringes. The second polyurethane tube was sealed using a nylon plug. The PVC column provides the necessary surface and support for the silicon tubes to be placed at specific depth. The pore-water samplers were installed in

May 2011 and replicates of 4 ml gas samples were extracted at 11 soil depths (5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 cm below the surface) monthly from July 2011. The gas samples extracted were transferred into pre-evacuated 4.5 ml exetainers (Labco ltd, High Wycombe, UK).

Figure 2.13: Pore-water equilibrators installed in temperate forested wetland to measure pore-water $CH_4$ concentrations at 11 soil depths (5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 cm below the surface).

### *2.4.2. Tropical forested wetland*

Pore-water samples were extracted at three soil depths (50, 100 and 150 cm below the soil surface) within the two study plots at two locations in the tropical forested wetland. The samplers were installed at the beginning of the experiment and were constructed of PVC pipes (3.2 cm diameter) 50, 100 and 150 cm long with holes drilled at one end to collect pore-water and capped using a lid at the other. During each measurement, a Teflon tubing (1.5 mm internal diameter) equipped with a three-way stopcock attached to the lid was used to extract pore-water, which was immediately transferred into a glass vial (12 ml) pre-purged with $N_2$.

*2.4.3. Mesocosm experiment*

Pore-water samplers were permanently installed into the mesocosms at the beginning of the experiment at three soil depths (10, 20 and 30 cm below the soil surface) and 2 ml of unfiltered pore-water was extracted monthly using a syringe applied with a prolonged suction pressure. Pore-water samplers were constructed using 0.64 cm polytetrafluoroethylene (PTFE) tubing (Cole-Parmer, London, UK) perforated with holes. The end of the tubing was blocked using silicone sealant and filled with glass wool (Plastipak, Becton Dickinson, Franklin Lakes, NJ, USA). The collected water samples were transferred into a glass vial (12 ml) pre-purged with $N_2$.

## 2.5. Methane sampling and analysis

Gas samples were extracted from the static chambers (t = 5, 20, 40, 60, 80 min for tree stems and t = 5, 15, 30, 45 min for peat surface) using a plastic syringe (30 ml) and transferred immediately into pre-evacuated 12 ml exetainers (Labco ltd, High Wycombe, UK). All gas samples were analysed for $CH_4$ within two weeks (temperate forested study) and four weeks (tropical forested study) after sampling. Methane concentrations from gas samples obtained *in situ* were determined using a cavity ring down laser spectroscopy (Los Gatos Research RMA-200 Fast Methane Analyser; Los Gatos Research, Mountain View, CA, USA) modified to employ the 'closed-loop' principle described by Baird *et al.* (2010) and outlined in Fig. 2.14. The minimum flux that could be detected by this method based upon instrument sensitivity and chamber volume was 0.4-3.5 $\mu g\ CH_4\ m^{-2}\ h^{-1}$.

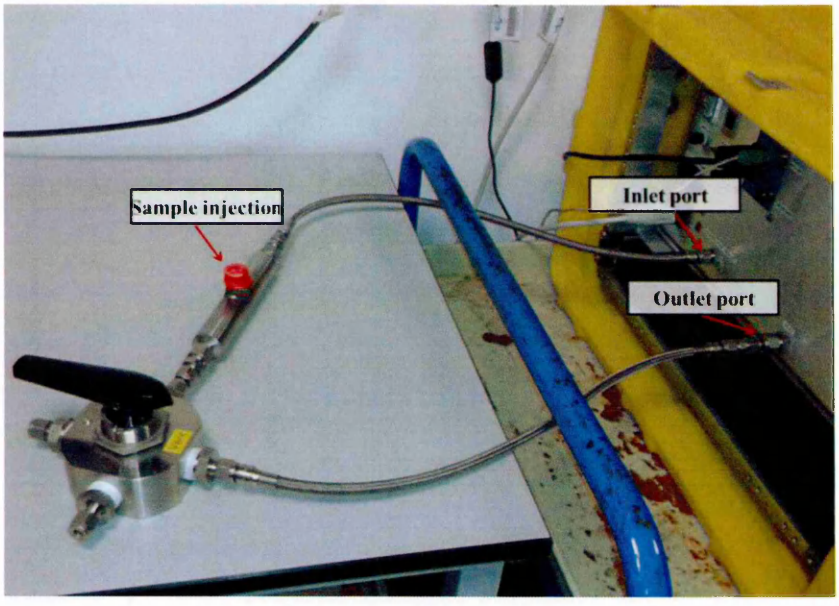


Figure 2.14: Modified CRDLS which includes an attachment loop constructed from a Swagelok double-ended miniature cylinder (50 $cm^3$), 0.5 μm particle filter, Swagelok 4-way ball valve, PTFE lined stainless steel braded hosing and Swagelok reducing unions, nuts and ferrules.

The CRDLS instrument uses an off-axis Integrated Cavity Output Spectroscopy (ICOS) and consists of a diode laser operating in the near-infrared, an optical cavity lined with reflective mirrors acting as an absorption cell and a photo-detector. A highly collimated laser beam tuned to a wavelength of 1653.723 nm beamed at a slight angle is reflected in the optical cavity containing reflective mirrors that creates a path length of *c.* 2500 m and the fractional absorption of laser beam at the $CH_4$ resonant wavelength is recorded by the detector, which is an absolute measure of the $CH_4$ concentration within the cavity. When $CH_4$ is introduced into the cavity, the intensity decay rate of the laser beam is reduced as a result of absorption.

In the mesocosm experiment, CRDLS system was used to quantify $CH_4$ emissions in real time. Each static chamber was fitted with inlet and outlet valves that were connected to the CRDLS using gas impermeable tubing. Gas from the chambers was circulated through the

analyser to perform real-time continuous measurements of $CH_4$ within the chamber (Fig. 2.15). A chamber closure time of 5 minutes was chosen for each measurement. The minimum flux that could be detected by this method based upon instrument sensitivity, chamber closure time and chamber volume was 0.1-0.3 μg $CH_4$ $m^{-2}$ $hr^{-1}$.

Figure 2.15: Static chambers connected to the CRDLS.

The gas and water samples extracted from the pore-water samplers were analysed using modified CRDLS after shaking the vials on a horizontal shaker for 5 minutes. Pore-water $CH_4$ concentrations were calculated using Henry's gas law as described by Blodau *et al.* (2007). All gas and water samples were analysed in duplicate.

## 2.6. Methane flux calculations

The rate of increase of $CH_4$ within the chamber was calculated from least squares linear regression analysis of concentration measurements versus time, the $CH_4$ emitting surface area and the volume of the chamber, and was converted to an appropriate measure of ecosystem flux. Fluxes initially obtained in μL $m^{-2}$ $s^{-1}$ were converted to mol $m^{-2}$ $s^{-1}$ using the Ideal Gas Law:

$$n = \frac{PV}{RT} \qquad \text{(Equation 2.1)}$$

Where n is the number of mole of analytical gas, P is the atmospheric pressure in atmospheres, V is the volume of the analyte, R is the ideal gas constant and T is the temperature in Kelvin.

The $R^2$ values were used for analysis of outliers. Samples that displayed $R^2 < 0.90$ with t = 0 concentrations being close to ambient concentrations (5% of data from hollows in tropical forested study and 12% of gas samples analysed for soil surfaces (vegetated and non-vegetated) in temperate forested study) were judged to represent natural ebullition events and were included when characterising ecosystem $CH_4$ fluxes.

## 2.7. Specific density of wood

Wood specific density was calculated for the wood samples extracted from both the temperate and tropical wetland trees. An increment borer (internal diameter = 5.1 mm, Haglöf Sweden AB, Långsele, Sweden) was used to extract wood samples at stem heights of 35, 75, 115 and 130 cm from the eight tree species (n = 4) in the tropical forested wetland and two tree species (26 of *Betula pubescens* and 20 of *Alnus glutinosa*) in the

temperate forested wetland. In both these forests, the wood samples were collected after the tree flux measurement campaign was concluded. Specific density of the wood was calculated based upon wood dry mass and volume (Williamson & Wiemann, 2010), a well-known technique used for over a decade. Wood volume was measured using a water displacement method (Archimedes principle) and wood dry weight by oven-drying the samples at 103°C for 48 h.

### 2.8. Ecosystem flux estimation

A relationship established between measured tree-stem $CH_4$ fluxes and corresponding stem sampling height for each species was used to estimate the stem-$CH_4$ fluxes along the length of the tree. Stem circumference also was measured at 10 cm intervals between 0 and 2 m height for trees studied within each study plots and was used to establish a relationship between stem height and circumference. This relationship was later applied to the entire length of the tree, and stem surface area of the tree was estimated by considering the tree as a truncated cone. Total $CH_4$ emissions along the length of each tree species was estimated by multiplying the $CH_4$ fluxes by the surface area (as estimated earlier) and the total number of trees per species. Tree-stem $CH_4$ flux per plot was estimated by dividing total stem emissions from all tree species, including tree species that did not emit $CH_4$ in the tropical forested wetland, and multiplying the resulting emissions per tree by the total number of trees. This approach assumes that a similar proportion of tree species and individual trees emitting and not emitting $CH_4$ are present in other areas of the forested wetland. The stem emission rates (2.5-10.6 mg $CH_4$ per tree $d^{-1}$) were used to estimate plot-level emissions in the tropical forested wetland. In the temperate forested wetland, however, $CH_4$ fluxes per plot for each month were estimated using the net $CH_4$ fluxes

measured in this study (monthly average) and the corresponding $CH_4$ emitting surface area. Therefore, the stem-$CH_4$ emission rates per tree varied monthly.

### 2.9. Statistical analysis

Statistical tests were performed using SPSS v.19 (SPSS, Chicago, IL, USA). All $CH_4$ fluxes were first tested for normality using Kolmogorov-Smirnov test and visual inspection of quantile-quantile plots followed by Shapiro-Wilk's test to test the level of significance ($P < 0.05$). The fluxes also were tested for equality of variance using Levene tests, where the Levene and Shapiro-Wilk's test was $P > 0.05$, parametric statistics such as general linear models were used and transformations were attempted where $P < 0.05$ for both tests. However, if the assumptions of normality and equality of variance were not met ($P < 0.05$) the variables were subjected to non-parametric tests such as Kruskal-Wallis and Mann-Whitney Tests. Statistical methods that are more specific to individual chapters are discussed within those chapters.

# CHAPTER THREE

## Controls on Methane Emissions from *Alnus glutinosa* Saplings

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### 3.1. Abstract


- Recent studies have confirmed significant tree-mediated $CH_4$ emissions in wetlands; however, factors and processes controlling such emissions are unclear. This study identifies the factors that control the emission of $CH_4$ from *Alnus glutinosa*.
- Methane fluxes from the soil surface, tree stem surfaces, leaf surfaces and whole-mesocosms, pore-water $CH_4$ concentrations and physiological factors (assimilation rate, stomatal conductance and transpiration) were measured from 4-year old *Alnus glutinosa* trees grown under two artificially controlled water-table positions.
- In the high water-table mesocosms up to 64% of $CH_4$ emitted was transported to the atmosphere through *Alnus glutinosa*. Stem emissions from 2 to 22 cm above the soil surface accounted for up to 42% of total tree-mediated $CH_4$ emissions. Methane emissions were not detected from leaves and no relationship existed between leaf surface

area and rates of tree-mediated $CH_4$ emissions. Tree stem-$CH_4$ flux strength was controlled by the amount of $CH_4$ dissolved in pore-water and the density of stem lenticels.

- This study identifies the principal mechanisms and controls on tree-mediated $CH_4$ emissions. The study further shows that stem surfaces dominate $CH_4$ egress from *Alnus glutinosa*, suggesting that leaf area index is not a suitable approach for scaling tree-mediated $CH_4$ emissions from all types of forested wetlands.

### 3.2. Introduction

Wetlands occupy *c.* 5% of the Earth's land area (Prigent *et al.,* 2007) and are the single largest natural source of $CH_4$ emissions to the atmosphere, representing 20-40% of the global $CH_4$ budget (Cicerone & Oremland, 1988; Denman *et. al.,* 2007). Methane produced by methanogenic *Archaea* in anoxic wetland sediment and soil (Conrad, 1989) is known to be released to the atmosphere via three pathways: pore-water diffusion, ebullition and transport through aerenchyma of herbaceous plants. However, there is growing evidence that woody plants represent a fourth pathway for emission of soil-produced $CH_4$ (Gauci *et al.,* 2010) - a pathway estimated to contribute up to 80 Tg $CH_4$ $a^{-1}$ globally to the atmosphere (Rice *et al.,* 2010).

Methane emission from the trunks of trees was first proposed by Schütz *et al.* (1991) and later confirmed by mesocosm experiments (Rusch & Rennenberg, 1998; Vann & Megonigal, 2003; Garnet *et al.,* 2005; Rice *et al.,* 2010) and field studies in forested wetlands (Terazawa *et al.,* 2007; Gauci *et al.*, 2010). These investigations have mostly confirmed that plant-mediated $CH_4$ emission is not limited to herbaceous plants but also is important in trees adapted to wet soil, because the latter facilitate $O_2$ supply to their roots

through the formation of aerenchymatous tissue, adventitious roots and hypertrophied lenticels (Megonigal & Day, 1992; Kozlowski, 1997). However, little is known at present about the factors and processes that control tree-mediated $CH_4$ emissions from wetlands. Evidence to date suggests that $CH_4$ transport in trees is driven mainly by diffusion and activated when soil $CH_4$ concentration exceeds atmospheric concentrations (Rusch & Rennenberg, 1998; Terazawa *et al.,* 2007). There is presently a lack of direct evidence for tree-mediated $CH_4$ transport via pressurised gas transport or transpiration, mechanisms which are known to drive $CH_4$ transport in a range of herbaceous plant species (e.g., Conrad, 1989; Grünfeld & Brix, 1999) and $CO_2$ transport in trees (e.g., Teskey & McGuire, 2005; McGuire *et al.,* 2007).

Only a few physiological and environmental factors (e.g., pore-water $CH_4$ concentration, atmospheric $CO_2$ concentration, stomatal conductance and leaf temperature) have been identified that influence tree-mediated $CH_4$ emissions (Vann & Megonigal, 2003; Garnet *et al.,* 2005) in contrast to herbaceous plant-mediated $CH_4$ emissions, which are known to be affected by a range of interacting biotic and abiotic factors (e.g., Whiting & Chanton, 1992, 1996; van Bodegom *et al.,* 2001; Megonigal *et al.,* 2004). In general, the factors that drive tree-mediated $CH_4$ emissions remain poorly understood, as do the relative contributions of stem and leaf surfaces to total $CH_4$ emissions from trees. Garnet *et al.* (2005) and Rice *et al.* (2010) expressed tree-mediated $CH_4$ emission rates as a function of leaf surface area and in the latter case, used leaf area index (LAI) to estimate tree-mediated $CH_4$ emissions at a global scale (Rice *at al.,* 2010). Other studies have expressed tree-mediated $CH_4$ emissions as a function of stem surface area (Rusch & Rennenberg, 1998; Terazawa *et al.,* 2007; Gauci *et al.,* 2010) although no study to date has quantified $CH_4$ emissions from stem lenticels. The capacity for lenticels to mediate $CH_4$ egress from trees

has explicitly been only assumed thus far because of their well-established role in stem aeration (e.g., McBain *et al.,* 2004).

This study investigated mechanisms of $CH_4$ emissions from *Alnus glutinosa* (common alder), a key wetland tree species inhabiting waterlogged soil throughout Europe. The study aimed to: i) evaluate the capacity of *Alnus glutinosa* to mediate $CH_4$ emissions, ii) determine the relative proportions of $CH_4$ transport through leaves and stems of *Alnus glutinosa*, and iii) establish the main factors that control $CH_4$ egress from *Alnus glutinosa*. This study tested the hypothesis that tree stems are the dominant means of $CH_4$ emission from wetland adapted trees and that fluxes are controlled by the supply of $CH_4$ to roots from the soil (pore-water concentration) and the presence of a 'means of escape' from the tree stem (lenticel density).

## 3.3. Materials and Methods

The study was conducted using 48 mesocosms, each containing a single *Alnus glutinosa* sapling. The mesocosms were divided into two treatments (24 each) based upon water-table position: one at the soil surface (HW) and the other 25 cm below the soil surface (LW). Further details on the mesocosms can be found in Chapter 2 (section 2.2.3)

### *3.3.1. Methane measurements*

Methane emission from the soil surface, stem surface (at two stem heights: 2-12 cm and 12-22 cm above the soil surface), leaf surface and the whole-mesocosm were measured using headspace static chambers at the peak of the growing season (12-13$^{th}$ and 24-25$^{th}$ of July and 6-7$^{th}$ and 20-21$^{th}$ of August 2011). On each measurement occasion, the following measurement order was followed: stem chamber (2-12 cm stem height), stem chamber (12-

22 cm stem height), leaf chamber, soil chamber and whole-mesocosm chamber. At the time measurements were conducted, average soil and air temperatures were 16.7 ± 0.06 °C and 26.5 ± 0.56 °C, respectively, average relative humidity was 63% ± 3.16%, and photosynthetically active radiation (PAR) was 1.85 ± 0.09 mol $m^{-2}$ $hr^{-1}$ (maximum PAR = 2.84 mol $m^{-2}$ $hr^{-1}$). Static chambers for measuring $CH_4$ flux from the soil, leaf, stem surfaces and whole-mesocosm are described in Chapter 2 (section 2.3.4).

Measurements were performed between 09:00 and 16:00 h on each sampling day, with emissions being measured from 12 trees per treatment on each day (24 trees in total). Changes in $CH_4$ concentrations in the static chambers were measured by cavity-ring down laser spectroscopy as described in Chapter 2 (section 2.5).

Diel patterns in $CH_4$ emission from the soil surface, whole-mesocosms and stem surfaces of *Alnus glutinosa* were investigated on 26 and 27 July 2011. Sampling was conducted during a 48-hr period in 4-hr sampling intervals (06:00-10:00, 10:00-14:00, 14:00-18:00, 18:00-22:00, 22:00-02:00 and 02:00-06:00 h), using the static headspace chambers as described in Chapter 2 (section 2.3.4). Six of the HW mesocosms were used, which contained *Alnus glutinosa* saplings that had a similar height, stem diameter and $CH_4$ emission rate from stems and soil. During diel measurements, the day and night air temperatures was 23.4 ± 0.98 and 15.7 ± 0.5 °C, respectively, but the soil temperature stayed relatively similar between day and night (16.4 ± 0.04 – 16 ± 0.06 °C).

### *3.3.2. Tree physiology measurements*

Net $CO_2$ assimilation, transpiration and stomatal conductance were measured from fully expanded leaves using a CIRAS-II portable photosynthesis system (PP Systems, MA, USA) and a Parkinson leaf chamber which enclosed 2.5 $cm^2$ of leaf surface area. During each sampling period, both leaf gas exchange and stem-$CH_4$ emissions were measured

simultaneously. Stem lenticel density was estimated using 2 × 2 cm grids on individual stems at two stem heights. The term 'stem lenticel density' represents only lenticels and not hypertrophied lenticels because the latter structures were not observed on trees from any of the HW mesocosms. Tree height, stem diameter, stem surface area, leaf surface area and number of branches and leaves were measured fortnightly. The stem surface area was estimated based upon stem circumference measured at intervals of 10 cm along the height of the tree and by considering the tree stem as a truncated cone. Branch surface area not enclosed within the leaf static chamber was also factored into stem surface area estimations. Leaf surface area of each branch was estimated using the product of the measured maximum width and length of 10-15 leaves per branch and a correction factor determined by estimating leaf surface area using graphing paper. Leaf surface area of each tree was then estimated using the leaf surface area determined per branch, and the number of branches and leaves per tree. Whole-tree photosynthesis, transpiration and stomatal conductance were estimated by multiplying the corresponding net fluxes with leaf surface area. (Please note: root growth, root density and root structure were not measured in this study).

### *3.3.3. Flux calculations*

Soil emissions were estimated by multiplying measured soil $CH_4$ fluxes by the soil surface area of each mesocosm after deducting tree basal area. Tree-mediated $CH_4$ emissions were estimated by subtracting soil emissions (as calculated above) from the measured whole-mesocosm $CH_4$ emissions. Tree emissions calculated by this approach were subsequently compared to $CH_4$ emissions measured using stem chambers (i.e., after establishing the relationship between stem emissions and stem height above the soil surface). Tree height

and the presence of branches prevented stem sampling at three heights above the soil surface in most cases and consequently, measurement of stem-$CH_4$ emissions from the 22-32 cm height interval was possible only for four trees. Relationships between stem height and rates of stem-$CH_4$ emissions established from these four trees were used to scale stem-$CH_4$ fluxes from the other trees where measurements were possible at only two height intervals.

For the trees with three stem chamber measurements, a power function relationship was observed between stem-$CH_4$ emission rate and stem sampling height for three of the four trees studied, which when used to estimate whole tree emissions, provided flux values that were very similar to tree-mediated $CH_4$ emissions estimated by subtracting soil emissions from whole-mesocosm $CH_4$ emissions (Table 3.1). One tree exhibited a linear relationship between stem-$CH_4$ flux and height of measurement; however, total tree $CH_4$ flux calculated using this relationship differed significantly from tree-mediated $CH_4$ emissions determined from whole mesocosm flux (Table 3.1). Therefore, $CH_4$ fluxes measured along the length of the tree stem were estimated using a regression model, which assumed tree stem-$CH_4$ emissions varied with height according to the power function relationship.

**Table 3.1:** Summary of mesocosm $CH_4$ fluxes (mg $hr^{-1}$ ± SE) for different emission pathways from *Alnus glutinosa* (n = 4) in high water-table treatment mesocosms. Stem $CH_4$ emissions were measured from three height intervals (2-12 cm, 12-22 cm and 22-32 cm above the soil surface).

| | Tree 1 | Tree 2 | Tree 3 | Tree 4 |
|---|---|---|---|---|
| | mg $hr^{-1}$ | | | |
| Stem $CH_4$ emissions at 2-12 cm stem height | 0.0171 ± 0.005 | 0.0294 ± 0.003 | 0.0225 ± 0.007 | 0.0358 ± 0.001 |
| Stem $CH_4$ emissions at 12-22 cm stem height | 0.0126 ± 0.001 | 0.0189 ± 0.002 | 0.0157 ± 0.002 | 0.0253 ± 0.001 |
| Stem $CH_4$ emissions at 22-32 cm stem height | 0.0096 ± 0.001 | 0.0142 ± 0.002 | 0.0130 ± 0.001 | 0.0172 ± 0.001 |
| Whole mesocosm $CH_4$ emissions | 0.181 ± 0.018 | 0.228 ± 0.013 | 0.192 ± 0.021 | 0.190 ± 0.014 |
| Total soil $CH_4$ emissions | 0.0701 ± 0.003 | 0.0942 ± 0.007 | 0.0792 ± 0.010 | 0.0915 ± 0.005 |
| Estimated total tree-mediated $CH_4$ emissions[a] | 0.111 ± 0.015 | 0.134 ± 0.006 | 0.112 ± 0.011 | 0.0986 ± 0.009 |
| Estimated total tree-mediated $CH_4$ emissions[b] | 0.104 ± 0.010[1] | 0.147 ± 0.021[2] | 0.138 ± 0.019[3] | 0.179 ± 0.014[4] |
| Estimated total tree-mediated $CH_4$ emissions[c] | 0.0466 ± 0.013[1] | 0.0681 ± 0.007[2] | 0.0628 ± 0.011[3] | 0.0862 ± 0.003[4] |

[a] Estimated by subtracting total soil $CH_4$ emissions from measured whole mesocosm $CH_4$ emissions.\

[b] $CH_4$ emissions measured along the length of *Alnus glutinosa* were estimated using a regression model, which assumed stem $CH_4$ emissions varied with height according to the power function relationship. Equations used: x = stem $CH_4$ emissions, mg $hr^{-1}$; y = stem height, cm. [1]($x = 39.5(y^{-0.42})$; $R^2 = 0.978$; $P < 0.001$); [2]($x = 83.8(y^{-0.534})$); $R^2 = 0.99$; $P < 0.0001$); [3]($x = 49.6(y^{-0.406})$); $R^2 = 0.99$; $P < 0.0001$); [4]($x = 102(y^{-0.522})$); $R^2 = 0.954$; $P < 0.01$).

[c] $CH_4$ emissions measured along the length of *Alnus glutinosa* were estimated using a regression model, which assumed stem $CH_4$ emissions varied with height according to the linear relationship. Equations used: x = stem $CH_4$ emissions, mg $hr^{-1}$; y = stem height, cm. [1]($x = 19.5y - 0.378$; $R^2 = 0.987$; $P < 0.001$); [2] ($x = 33.7y - 0.760$; $R^2 = 0.952$; $P < 0.001$); [3]($x = 30.3y - 0.664$; $R^2 = 0.97$; $P < 0.001$); [4]($x = 42.1y - 0.934$; $R^2 = 0.996$; $P < 0.0001$)

*3.3.4. Statistical analyses*

Methane fluxes are reported as the overall means of fortnightly measurements conducted in July and August, 2011 (± SE). Statistical analyses were conducted using SPSS software v.19 (SPSS, Chicago, IL, USA) and a significance level of $P < 0.05$. All datasets were tested for normal distribution using Shapiro-Wilk's test and homogeneity of variance using Levene's test. For each fortnightly measurement, cumulative $CH_4$ fluxes were calculated and high and low water-table treatments were compared using repeated measures ANOVA. Variations between HW and LW mesocosm $CH_4$ emissions and stem (at both stem heights), soil emissions over time and diel variations in $CH_4$ fluxes over 48-hr period were tested using a general linear model (ANOVA repeated measures). Tukey's HSD test ($P \leq 0.05$) was used for comparison of means. Relationships between whole-mesocosm $CH_4$ emissions, stem-$CH_4$ fluxes, whole-tree assimilation, stomatal conductance, transpiration, stem diameter, leaf surface area, pore-water $CH_4$ concentration, stem lenticel density, PAR, air and soil temperature were evaluated using regression models. Regression models were also used to evaluate relationships between stem $CH_4$ fluxes, whole mesocosm $CH_4$ emissions and independent variables measured during the diel variation experiment. The relative contributions of all the independent variables measured (whole-tree assimilation, stomatal conductance, transpiration, stem diameter, leaf surface area, pore-water $CH_4$ concentration, stem lenticel density, PAR, air and soil temperature) to stem-$CH_4$ emissions and whole-mesocosm $CH_4$ emissions were determined using stepwise multiple regression analysis. All independent variables were first tested for multi-collinearity and homoscedasticity. Pore-water $CH_4$ concentration at 20 and 30 cm soil depth was highly correlated ($R = 0.97$), hence pore-water $CH_4$ concentration at 30 cm below the soil surface was excluded from the stepwise multiple regression analysis. A weak correlation was

observed between stem diameter and stem lenticel density ($R$ = 0.42) and therefore both the variables were included in the stepwise multiple regression analysis.

## 3.4. Results

The trees grown under HW conditions developed visible morphological features, including leaf chlorosis, leaf abscission, formation of adventitious roots, stem thickening and increased number of stem lenticels within three weeks of transplanting. The density of lenticels in July 2011 in the HW treatment trees was 1.67 ± 0.1 $cm^{-2}$ (between 2-22 cm stem height) compared to 0.85 ± 0.3 $cm^{-2}$ in trees grown under LW conditions.

### *3.4.1. Mesocosm $CH_4$ emissions*

Throughout the observation period, average soil $CH_4$ flux and stem-$CH_4$ flux from LW mesocosms were significantly different ($P < 0.001$) from HW mesocosms. The average soil $CH_4$ flux rate from HW mesocosms was 0.78 ± 0.02 mg $m^{-2}$ $hr^{-1}$, which was significantly larger ($P < 0.001$) than fluxes from LW mesocosms (−5.31 ± 0.48 $\times 10^{-3}$ mg $m^{-2}$ $hr^{-1}$) where only $CH_4$ uptake occurred at the soil surface (Fig. 3.1). Tree stems also did not emit $CH_4$ in the LW mesocosms and $CH_4$ emissions from leaves were not detected in either the LW or HW mesocosms (i.e., the change in $CH_4$ concentration in leaf flux chamber was below the instrument detection limit of *c.* 2 ppbv).

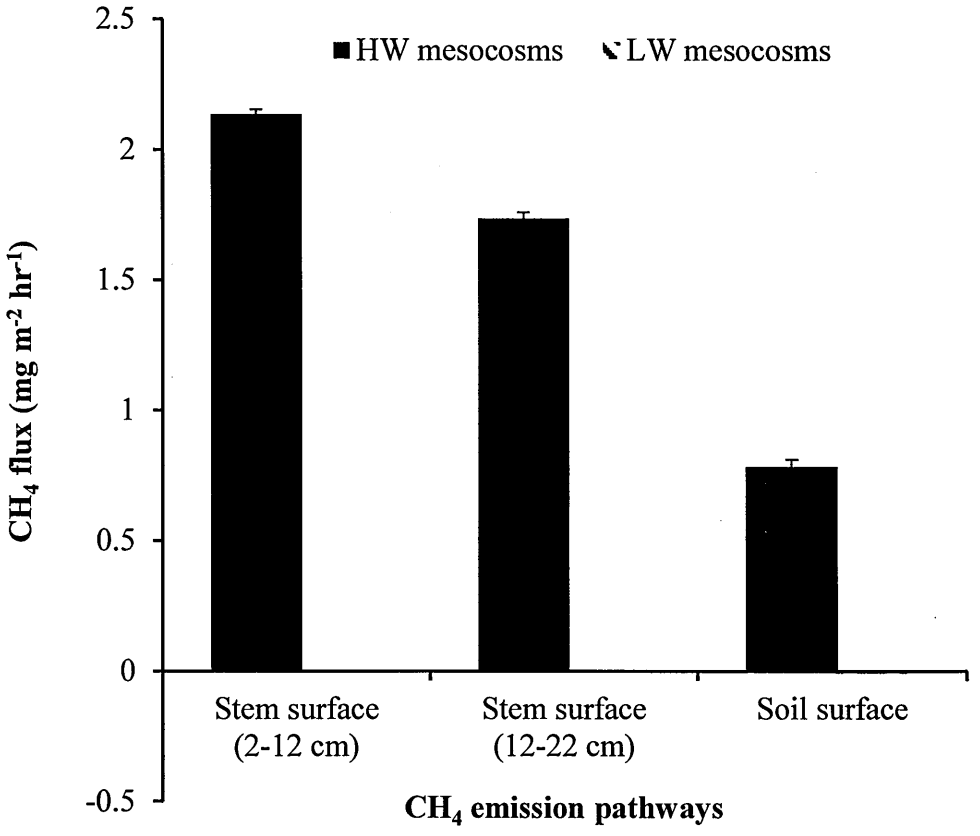


Figure 3.1: Average $CH_4$ fluxes measured in the HW and LW mesocosms (n = 24) during the observation period July and August 2011. Bars represent $CH_4$ fluxes measured from stem surfaces at two stem heights at 2-12 and 12-22 cm height above the soil surface (expressed per stem unit area) and the soil surface (expressed per soil unit area). Error bars represent the mean ± SE.

In HW mesocosms, rates of stem-$CH_4$ flux (expressed per stem unit area) were significantly larger than soil $CH_4$ fluxes ($P < 0.01$; Fig. 3.1). Stem-$CH_4$ fluxes (2-22 cm stem height) averaged 1.94 ± 0.06 mg $m^{-2}$ $hr^{-1}$ compared to average soil $CH_4$ emission rates of 0.78 ± 0.02 mg $m^{-2}$ $hr^{-1}$. Stem-$CH_4$ fluxes measured at each individual stem heights (2-12 cm and 12-22 cm above the soil surface) were larger than soil $CH_4$ fluxes in all the HW mesocosms (Fig. 3.1). Mean $CH_4$ fluxes at 2-12 cm stem height were significantly larger than fluxes at 12-22 cm stem height during both July and August. Rates of $CH_4$ flux from soil exhibited minimal variation between the different HW mesocosms (0.694 - 0.948 mg $m^{-2}$ $hr^{-1}$) but there were significant variations in stem-$CH_4$ flux rate at both stem sampling

heights (1.39-2.72 mg $m^{-2}$ $hr^{-1}$ at 2-12 cm height and 1.27-2.38 mg $m^{-2}$ $hr^{-1}$ at 12-22 cm height). Both soil and stem-$CH_4$ fluxes measured in the HW mesocosms were greater than $CH_4$ emission rates reported for *in situ* forested wetland ecosystems where both sources were measured (Terazawa *et al.,* 2007; Gauci *et al.,* 2010) most likely due to elevated concentrations of acetate in the mesocosm supply water, which would have stimulated soil methanogenesis.

The mean contributions of $CH_4$ flux from *Alnus glutinosa* and the soil surface to whole-mesocosm emission were 0.121 ± 0.0046 mg $hr^{-1}$ $mesocosm^{-1}$ and 0.077 ± 0.0023 mg $hr^{-1}$ $mesocosm^{-1}$, respectively (Table 3.2). Approximately 61 ± 3% of $CH_4$ emissions from the mesocosms resulted from transport through *Alnus glutinosa.* The remaining 39 ± 3% of $CH_4$ flux was released from the soil surface with transport occurring most likely via diffusion through pore-water (Table 3.2). Ebullition was not detected from any of the mesocosms during flux measurements. Tree stems between 2 and 22 cm height above the soil surface released approximately 37 ± 5% of total tree-mediated $CH_4$ flux (Table 3.2).

Table 3.2: Summary of mesocosm $CH_4$ fluxes (mg $hr^{-1}$ ± SE) for different emission pathways in the HW mesocosms.

| | HW mesocosms | Percentage contribution |
|---|---|---|
| | mg $hr^{-1}$ | % |
| Total soil $CH_4$ emissions | 0.077 ± 0.0033 | 39 ± 3[c] |
| Estimated total tree-mediated $CH_4$ emissions[a] | 0.121 ± 0.0036 | 61 ± 3[c] |
| Whole-mesocosm $CH_4$ emissions | 0.197 ± 0.0069 | 100[c] |
| Estimated total tree-mediated $CH_4$ emissions[b] | 0.139 ± 0.0038 | 71 ± 3.8[c] |
| Stem-$CH_4$ emissions at 2-12 cm stem height | 0.026 ± 0.0029 | 22 ± 2.7[d] |
| Stem-$CH_4$ emissions at 12-22 cm stem height | 0.0185 ± 0.0028 | 15 ± 2.3[d] |

[a] Estimated by subtracting total soil $CH_4$ emissions from measured whole-mesocosm $CH_4$ emissions.

[b] $CH_4$ emissions measured along the length of the tree were estimated using a regression model, which assumed stem-$CH_4$ emissions varied with height according to the power function relationship as described in materials and methods section 3.3.3.

[c] Percentage contributions to whole-mesocosm $CH_4$ emissions.

[d] Percentage contributions to total tree-mediated $CH_4$ emissions estimated using [a] (subtracting total soil $CH_4$ emissions from measured whole-mesocosm $CH_4$ emissions).

### *3.4.2. Controls on tree-mediated $CH_4$ emissions*

During the diel flux experiment (i.e., 48-hr measurement campaign), no relationship was observed between light levels and whole-mesocosm $CH_4$ emissions or directly measured stem-$CH_4$ fluxes (Fig. 3.2). Methane emissions from stems at two heights (Fig. 3.2) and whole-mesocosms showed no marked diel variation ($P > 0.05$). Day and night $CH_4$ emissions from the whole-mesocosm averaged 0.19 ± 0.011 and 0.17 ± 0.01 mg $h^{-1}$ $mesocosm^{-1}$, respectively (a difference of 10.5%; Table 3.3; although not statistically significant ($P > 0.05$).

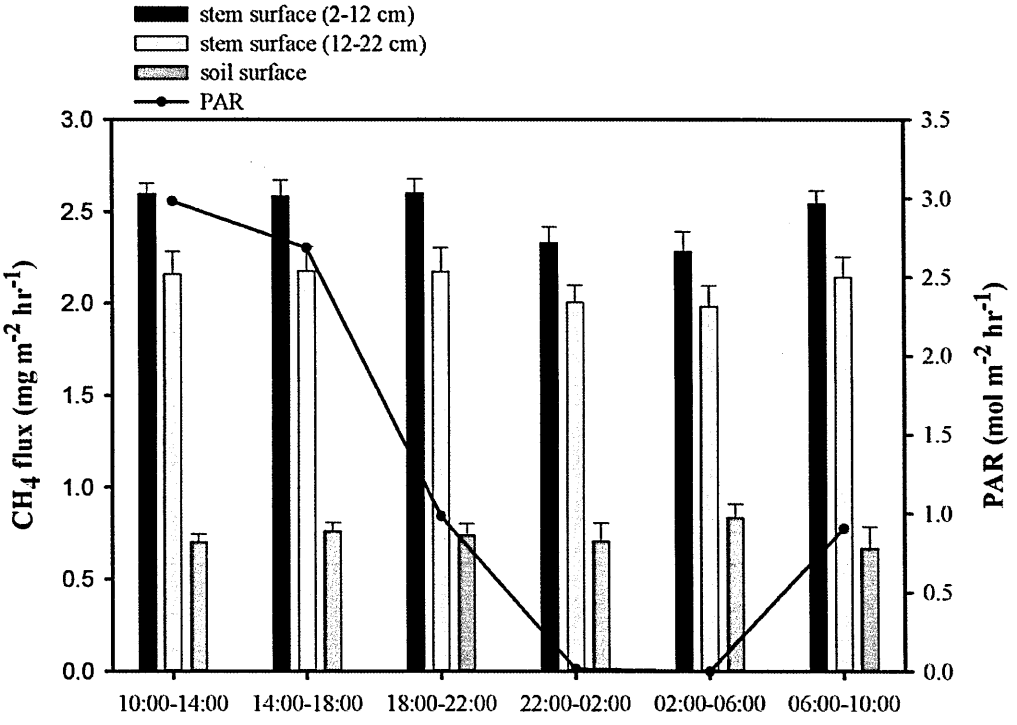


Figure 3.2: Average $CH_4$ fluxes measured over a 48-hr day cycle (n = 6). Bars represent $CH_4$ fluxes measured from stem surfaces at 2-12 cm and 12-22 cm height above the soil surface (expressed per stem unit area) and soil surface (expressed per soil unit area). Error bars represent the mean ± SE.

Table 3.3: Rates of $CH_4$ flux (mg $hr^{-1}$ ± SE) from *Alnus glutinosa* trees (n = 6) measured during the day and at night. Day and night time data represents the mean of measurements performed between 10:00 and 18:00 and 22:00 and 06:00, respectively.

| | Day | Night | Percentage difference |
|---|---|---|---|
| | (mg $hr^{-1}$ $mesocosm^{-1}$) | | (%) |
| Tree-mediated $CH_4$ emissions[a] | 0.112 ± 0.0063 | 0.098 ± 0.0056 | 13% |
| Stem height (2-12 cm) | 0.0274 ± 0.0012 | 0.0245 ± 0.0011 | 11% |
| Stem height (12-22 cm) | 0.023 ± 0.009 | 0.0211 ± 0.0010 | 9% |
| Whole-mesocosm $CH_4$ emissions | 0.19 ± 0.011 | 0.17 ± 0.01 | 10.5% |

[a] Estimated by subtracting total soil $CH_4$ emissions from whole-mesocosm $CH_4$ emission.

Air temperature rose rapidly in the morning both days during the diel experiment, reaching a maximum of 27.5 °C by 13:00. Soil temperature remained relatively constant (16.4 ± 0.04 – 16 ± 0.06 °C; day and night temperature). Weak relationships were observed between some of the measured variables (air and soil temperature, whole-tree stomatal conductance and transpiration) and stem and whole-mesocosm $CH_4$ emissions (Table 3.4).

Table 3.4: Relationships between stem-$CH_4$ emissions (mg $m^{-2}$ $hr^{-1}$), whole mesocosm $CH_4$ emissions (mg $hr^{-1}$ $mesocosm^{-1}$) and measured variables during a 24-hr day-night cycle (n = 6).

| Measured variables | Range | Relationship between 2-12 cm stem-$CH_4$ emissions and variable ($R^2$) | Relationship between 12-22 cm stem-$CH_4$ emissions and variable ($R^2$) | Relationship between whole-mesocosm $CH_4$ emissions and variable ($R^2$) |
|---|---|---|---|---|
| Whole-tree assimilation (mmol $hr^{-1}$) | 24.3 ± 3.61 | y = 0.0033x + 2.41 (0.09) | y = 0.0037x + 2.02 (0.08) | y = 0.0003x + 0.175 (0.05) |
| Whole-tree stomatal conductance (mol $hr^{-1}$) | 295 ± 28.9 | y = 0.0004x + 2.37 (0.16)* | y = 0.0004x + 1.99 (0.10) | y = 0.00002x + 0.1745 (0.05) |
| Whole-tree transpiration (mol $hr^{-1}$) | 2.54 ± 0.41 | y = 0.0307x + 2.41 (0.11)* | y = 0.046x + 1.99 (0.16)* | y = 0.0014x + 0.177 (0.02) |
| PAR (mol $m^{-2}$ $hr^{-1}$) | 1.21 ± 0.21 | y = 0.044x + 2.44 (0.06) | y = 0.045x + 2.05 (0.04) | y = 0.0043x + 0.176 (0.05) |
| Soil temperature (°C) | 16.2 ± 0.04 | y = 0.292x - 2.25 (0.14)* | y = 0.181x - 0.831 (0.03) | y = 0.0277x - 0.267 (0.11)* |
| Air temperature (°C) | 19.3 ± 0.67 | y = 0.0216x + 2.08 (0.14)* | y = 0.015x + 1.82 (0.05) | y = 0.002x + 0.143 (0.10) |

* $P < 0.05\%$.

During the fortnightly measurements conducted between 09:00 and 16:00 h whole-tree stomatal conductance and assimilation ranged from 276-717 mol $hr^{1}$ and 28.6-70 mmol $hr^{-1}$, respectively, with maximum rates observed between 12:00 to 14:00 h. However, stem-$CH_4$ emissions did not peak in this period consistent with the results of diel flux experiment (Fig. 3.2) which exhibited no significant relationship with time of day. No significant relationships were observed between stem and whole-mesocosm $CH_4$ emission rates and leaf physiological factors (i.e., whole-tree stomatal conductance, assimilation and transpiration; Tables 3.5 and 3.6). Similarly, leaf surface area also did not display any relationship with variations in stem or whole-mesocosm $CH_4$ emission rates, nor did PAR or soil and air temperature (Tables 3.5 and 3.6).

Pore-water $CH_4$ concentration varied with depth in HW mesocosms, with the highest levels measured at 20 and 30 cm below the peat surface, averaging 786 ± 16.2 μmol $l^{-1}$ and 778 ± 15.4 μmol $l^{-1}$, respectively (Table 3.5). A positive linear relationship was observed between stem-$CH_4$ emissions measured at 2-12 cm height and pore-water $CH_4$ concentrations at 20 cm soil depth ($R^2 = 0.52$; Table 3.5) and 30 cm ($R^2 = 0.57$; Table 3.5) in all HW mesocosms. Similar relationships also were observed between pore-water $CH_4$ concentration at both soil depths and stem emissions measured at 12-22 cm height (Table 3.5) and whole-mesocosm emissions (Fig. 3.3a; Table 3.6).

Table 3.5: Relationships between stem-$CH_4$ emissions (mg $m^{-2}$ $hr^{-1}$) and measured variables between 09:00 and 16:00 during the observation period July and August, 2011.

| Measured variable | Range | Relationship between 2-12 cm stem-$CH_4$ emissions and variable ($R^2$) | Relationship between 12-22 cm stem-$CH_4$ emissions and variable ($R^2$) |
|---|---|---|---|
| Pore-water $CH_4$ concentrations ($\mu$mol $l^{-1}$) | 10 cm below the soil surface | | |
| | 693 ± 12.1 | y = 0.0038x - 0.516 (0.39) *** | y = 0.0034x - 0.608 (0.41) *** |
| | 20 cm below the soil surface | | |
| | 786 ± 16.2 | y = 0.0033x - 0.483 (0.52) *** | y = 0.0023x - 0.0328 (0.32) ** |
| | 30 cm below the soil surface | | |
| | 778 ± 15.4 | y = 0.0037x - 0.723 (0.57) *** | y = 0.0024x - 0.165 (0.34) ** |
| Stem lenticel density (lenticels $cm^{-2}$) | 1.90 ± 0.12[a]<br>1.45 ± 0.10[b] | y = 0.563x[a] + 1.0631 (0.77) *** | y = 0.540x[b] + 0.954 (0.71) *** |
| Stem diameter at the base (cm) | 4.17 ± 0.03 | y = 0.996x - 2.02 (0.22)* | y = 0.749x - 1.39 (0.16)* |
| Whole-tree assimilation (mmol $hr^{-1}$) | 51.4 ± 2.14 | y = -0.014x + 2.83 (0.12) | y = -0.0039x + 1.93 (0.02) |
| Whole-tree stomatal conductance (mol $hr^{-1}$) | 510 ± 22 | y = -0.0003x + 2.27 (0.006) | y = -0.0007x + 2.09 (0.06) |
| Whole-tree transpiration (mol $hr^{-1}$) | 4.06 ± 0.26 | y = 0.048x + 1.93 (0.02) | y = 0.043x + 1.56 (0.03) |
| Leaf surface area ($m^2$) | 1.08 ± 0.04 | y = -0.525x + 2.69 (0.09) | y = -0.464x + 2.23 (0.09) |
| PAR (mol $m^{-2}$ $hr^{-1}$) | 1.85 ± 0.09 | y = -0.210x + 2.52 (0.07) | y = -0.119x + 1.96 (0.03) |
| Air temperature (°C) | 26.5 ± 0.56 | y = -0.037x + 3.11 (0.08) | y = -0.017x + 2.17 (0.02) |
| Soil temperature (°C) | 16.7 ± 0.06 | y = -0.038x + 2.77 (0.0008) | y = -0.097x + 3.35 (0.007) |

* $P < 0.05\%$ ; ** $P < 0.01\%$ ; *** $P < 0.001\%$ ; [a] stem lenticel density measured at 2-12 cm height above the soil surface; [b] stem lenticel density measured at 12-22 cm height above the soil surface.

Table 3.6: Relationship between whole-mesocosm $CH_4$ emissions (mg $hr^{-1}$ $mesocosm^{-1}$) and measured variables between 09:00 and 16:00 during the observation period July and August, 2011.

| Measured variable | Range | Relationship between whole-mesocosm $CH_4$ emissions and variables ($R^2$) |
|---|---|---|
| Pore-water $CH_4$ concentrations ($\mu mol\ l^{-1}$) | 10 cm below the soil surface | |
| | 693 ± 12.1 | y = 0.0002x + 0.033 (0.31) ** |
| | 20 cm below the soil surface | |
| | 786 ± 16.2 | y = 0.0002x + 0.024 (0.48) *** |
| | 30 cm below the soil surface | |
| | 778 ± 15.4 | y = 0.0002x + 0.017 (0.48) *** |
| Stem lenticel density (lenticels $cm^{-2}$) | 1.67 ± 0.10[a] | y = 0.042x + 0.127 (0.69) *** |
| Stem diameter at the base (cm) | 4.17 ± 0.03 | y = 0.051x - 0.016 (0.11) |
| Whole-tree assimilation ($mmol\ hr^{-1}$) | 51.4 ± 2.14 | y = -0.0002x + 0.205 (0.004) |
| Whole-tree stomatal conductance ($mol\ hr^{-1}$) | 510 ± 22 | y = -0.00003x + 0.210 (0.01) |
| Whole-tree transpiration ($mol\ hr^{-1}$) | 4.06 ± 0.26 | y = 0.0027x + 0.186 (0.02) |
| Leaf surface area ($m^{-2}$) | 1.08 ± 0.04 | y = -0.029x + 0.2629 (0.06) |
| PAR ($mol\ m^{-2}\ hr^{-1}$) | 1.85 ± 0.09 | y = -0.0121x + 0.22 (0.05) |
| Air temperature (°C) | 26.5 ± 0.56 | y = -0.003x + 0.282 (0.11) |
| Soil temperature (°C) | 16.7 ± 0.06 | y = 0.0131x - 0.021 (0.02) |

** $P < 0.01\%$; *** $P < 0.001\%$; [a] stem lenticel density measured between 2-22 cm height above the soil surface.

Although stem and whole-mesocosm $CH_4$ emissions increased at higher pore-water $CH_4$ concentration, the data suggest that controls other than soil $CH_4$ concentration are important in determining variations in stem-$CH_4$ emission rates when water-table levels are situated close to the surface. Stem diameter variations between the trees were minimal, averaging 4.17 ± 0.03 cm and only a weak relationship existed between stem diameter and stem-$CH_4$ emissions at both measurement heights (Table 3.5). However, significant positive linear relationships were observed between rates of stem-$CH_4$ flux and stem lenticel density in the HW mesocosms for both the 2-12 cm ($R^2 = 0.77$; $P < 0.001$; Table 3.5; Fig. 3.4a) and 12-22 cm ($R^2 = 0.71$; $P < 0.001$; Table 3.5; Fig. 3.4b) stem height intervals. A similar relationship also was observed between whole-mesocosm $CH_4$ emission rates and stem lenticel density measured between 2-22 cm stem height (Table 3.6; Fig. 3.3b).

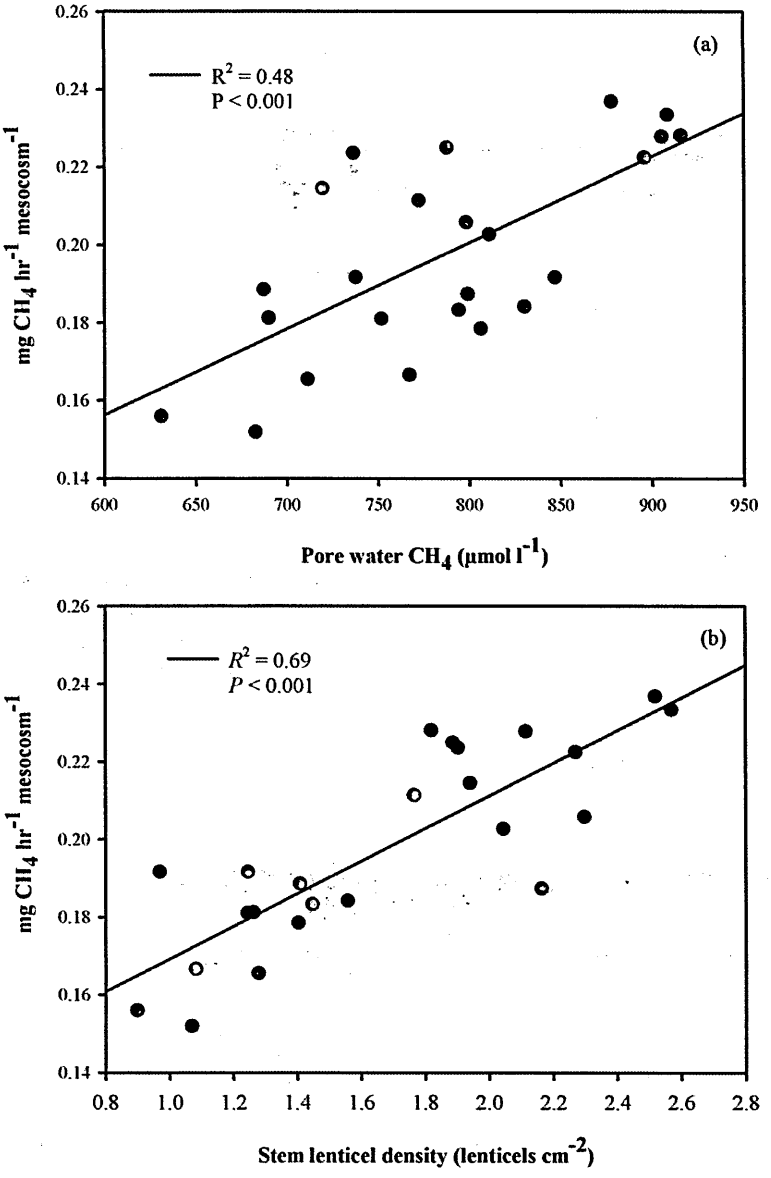


Figure 3.3: The relationship between whole-mesocosm $CH_4$ emissions and (a) pore-water $CH_4$ concentrations measured at 20 cm soil depth and (b) stem lenticel density at 2-22 cm of height above the soil surface during the observation period July and August 2011. The regression equations are: (a) y = 0.0002 × (pore-water $CH_4$ concentration) + 0.024; and (b) y = 0.042 × (stem lenticel density) + 0.127.

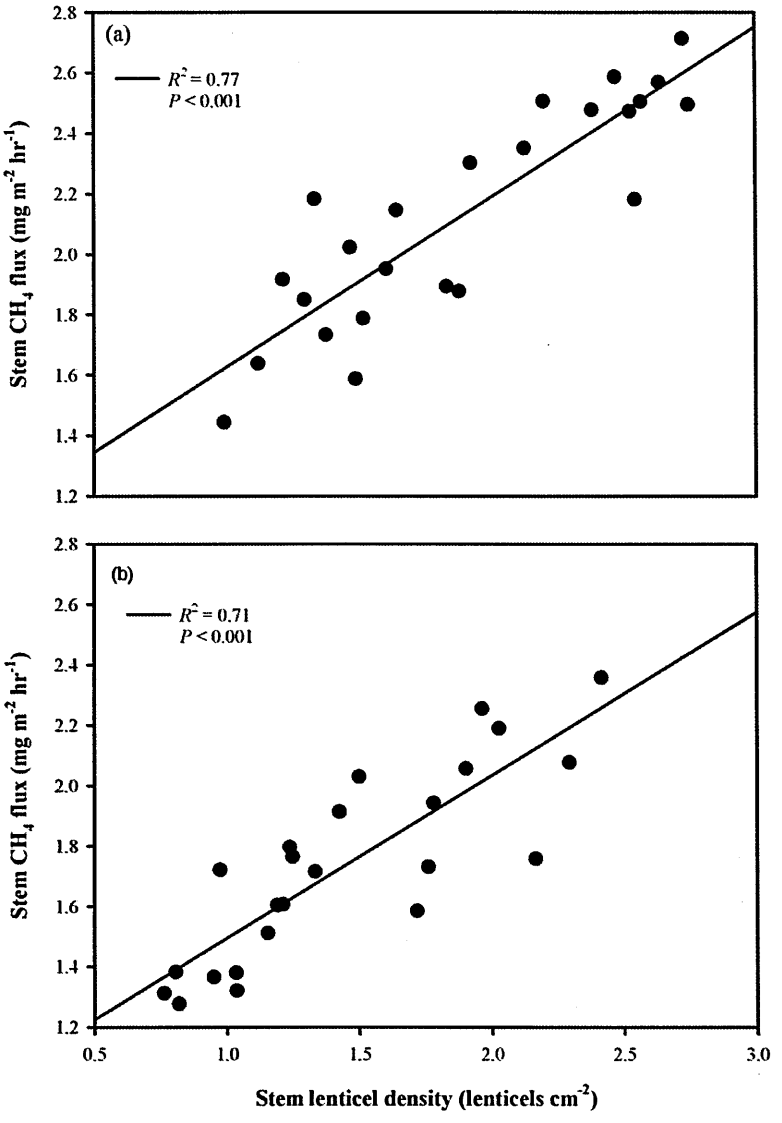


Figure 3.4: The relationship between stem-$CH_4$ emissions and stem lenticel density at a) 2-12 cm height and b) 12-22 cm height above the soil surface measured in July and August 2011. The regression equations are: (a) y = 0.563 × (stem lenticel density) + 1.0631; and (b) y = 0.540 × (stem lenticel density) + 0.954.

Stepwise multiple linear regressions on data pooled from the HW mesocosms show that pore-water $CH_4$ concentration at 20 cm soil depth ($P = 0.004$) and stem lenticel density at 2-12 cm stem height ($P < 0.001$) contributed significantly to differences in stem-$CH_4$ emissions, collectively accounting for 84% ($P < 0.001$) of the variation (Table 3.7). Stepwise multiple regression analysis also suggested that approximately 79% ($P < 0.001$) of variation in whole-mesocosm $CH_4$ emissions was explained by differences in the concentration of $CH_4$ dissolved in pore-water at 20 cm soil depth ($P = 0.002$) and lenticel density between 2-22 cm stem height ($P < 0.0001$; Table 3.7). Equations for estimating stem $CH_4$ emissions at 2-12 cm stem height and whole-mesocosm $CH_4$ emissions as a function of pore-water $CH_4$ concentration at 20 cm soil depth (X) and lenticel density (between 2-12 cm and 2-22 cm stem height for stem-$CH_4$ emissions and whole-mesocosm $CH_4$ emissions, respectively) (Y) obtained using stepwise multiple regressions are:

Stem-$CH_4$ emissions = 0.002 (X) + 0.377 (Y) + 0.026

Whole-mesocosm $CH_4$ emissions = 0.00013 (X) + 0.031 (Y) + 0.047

Table 3.7: Results of stepwise multiple regression analysis of stem-$CH_4$ emissions at two stem height positions (2-12 and 12-22 cm above the soil surface) and whole-mesocosm $CH_4$ emissions and all the independent variables measured during this study.

| | Stem-$CH_4$ emissions (2-12 cm) | | Stem-$CH_4$ emissions (12-22 cm) | | Whole-mesocosm $CH_4$ emissions | |
|---|---|---|---|---|---|---|
| | Coefficients | Standard Error | Coefficients | Standard Error | Coefficients | Standard Error |
| Adjusted $R^2$ | 0.835 ($P = 0.0002$) | | 0.797 ($P = 0.0002$) | | 0.785 ($P = 0.0008$) | |
| Constant | 0.026 ($P = 0.939$) | 0.335 | 0.172 ($P = 0.496$) | 0.248 | 0.047 ($P = 0.069$) | 0.025 |
| Pore-water $CH_4$ concentrations 20 cm below the soil surface ($\mu$mol $l^{-1}$) | 0.002 ($P = 0.004$) | 0.001 | 0.001 ($P = 0.003$) | 0.0001 | 0.00013 ($P = 0.002$) | 0.00004 |
| Stem lenticel density (lenticels $cm^{-2}$) | 0.377 ($P = 0.0001$) | 0.79 | 0.429 ($P < 0.0001$) | 0.069 | 0.031 ($P < 0.0001$) | 0.006 |

## 3.5. Discussion

### *3.5.1. Methane emission from Alnus glutinosa*

Results demonstrate that stem-$CH_4$ emissions are a major pathway for $CH_4$ egress in *Alnus glutinosa* from the HW mesocosms and that stem surfaces are responsible for most of the tree-mediated $CH_4$ emissions (Fig. 3.1; Table 3.2). Approximately 61% of the HW mesocosm $CH_4$ emissions resulted from $CH_4$ venting to the atmosphere through *Alnus glutinosa* (Table 3.2). The relative contribution of tree-mediated $CH_4$ emissions to ecosystem emissions, however, may vary in natural wetlands depending on factors such as tree species, stand density, height, stem diameter, water table depths and the area of soil surface emitting $CH_4$, and thus should be assessed *in situ.*

Methane emissions from leaf surfaces of *Alnus glutinosa* were not detected but large emissions were measured from stem surfaces, consistent with previous studies by Rusch & Rennenberg (1998) and Gauci *et al.* (2010). These findings collectively suggest that stem surfaces are the principal point of $CH_4$ egress from *Alnus glutinosa.* Notably, Garnet *et al.* (2005) and Rice *et al.* (2010) reported tree-mediated $CH_4$ emission rates as a function of leaf surface area, suggesting that leaves may be a factor in $CH_4$ transport through *Taxodium distichum, Fraxinus latifolia, Populus trichocarpa* and *Salix fluviatilis.* These contrasting observations may be the result of differences in tree species in anatomical, morphological and physiological characteristics (*Alnus glutinosa* vs. *Taxodium distichum, Fraxinus latifolia, Populus trichocarpa* and *Salix fluviatilis*), gas transport mechanisms (i.e., molecular diffusion vs. pressurised $CH_4$ transport) and development stage (i.e., leaves may be the principal surface of $CH_4$ egress in younger seedlings due to smaller stem surface area). Nevertheless, the absence of $CH_4$ emissions from the leaf surfaces of *Alnus glutinosa* and lack of a relationship between leaf surface area and stem or whole-

mesocosm $CH_4$ emissions (Tables 3.5 and 3.6) suggest that LAI is not a suitable scaling metric for estimating tree-mediated $CH_4$ emissions from all types of forested wetland.

### *3.5.2. Controls on tree-mediated $CH_4$ emissions*

The uptake of $CH_4$ by soil and absence of stem-$CH_4$ emissions in the LW mesocosms and significant $CH_4$ fluxes from the HW mesocosms indicate that water-table level was a dominant control on $CH_4$ production and release. This finding is consistent with the longstanding view that water-table position strongly regulates soil $CH_4$ production and consumption in wetlands (Grünfeld & Brix, 1999 and references within). Notably, variations in stem-$CH_4$ emissions in the mesocosms were largely independent of soil and air temperature, which provides an opportunity to evaluate other variables that may influence rates of stem-$CH_4$ flux.

During fortnightly measurements in the HW mesocosms some variables controlled stem and whole-mesocosm $CH_4$ emissions more strongly than others. Leaf surface area, whole-tree transpiration, assimilation and stomatal conductance did not display a significant relationship with stem and whole-mesocosm $CH_4$ emissions. However, stem diameter at the base explained up to 22% of emission variations (Tables 3.5 and 3.6). Pore-water $CH_4$ concentration and stem lenticel density exhibited strong relationships with stem-$CH_4$ emissions (Table 3.5) and collectively explained up to 84% of variation in emission rates (Table 3.7). Stem lenticel density, in particular, strongly influenced stem and whole-mesocosm $CH_4$ emission rates (Figs. 3.3b and 3.4; Tables 3.5, 3.6 and 3.7). These findings suggest that variations in tree-mediated $CH_4$ emissions are controlled primarily by differences in pore-water $CH_4$ concentration and the number of stem lenticels per unit area on wetland-adapted trees.

Transport of soil-produced gases (i.e., $N_2O$ and $CH_4$) from the root zone through plant aerenchyma followed by release to the atmosphere through stem surfaces generally is attributed to lenticels because of their well understood role in aerating stems (e.g., Rusch & Rennenberg, 1998; McBain *et al.,* 2004). The strong positive linear relationship observed between stem-$CH_4$ emissions, whole-mesocosm $CH_4$ emissions and stem lenticel density suggests that the number of stem lenticels exerts an important control over rates of stem-$CH_4$ flux (Figs. 3.3b and 3.4; Tables 3.5, 3.6 and 3.7), confirming the importance of these adaptive structures as exit points for $CH_4$ egress from flood-tolerant trees (Rusch & Rennenberg, 1998; Purvaja *et al.,* 2004; Terazawa *et al.,* 2007). This finding has particular significance because formation of lenticels on stems, roots and root nodules, including hypotrophied lenticels, has been reported in many flood tolerant trees (Kozlowski, 1997), including on aerial roots, knees and pneumatophores of mangroves and *Taxodium distichum* (Pulliam, 1992; Purvaja *et al.,* 2004).

While this study demonstrates a strong positive relationship between stem lenticel density and tree-mediated $CH_4$ emissions in *Alnus glutinosa*, further work is required to determine whether such a relationship is common in other tree species. Lenticel presence, number, type, degree of opening, development stage and area vary between tree species (Langenfeld-Heyser, 1997; Kalachanis & Psaras, 2007). Moreover, the development stage of a tree species (Lendzian, 2006; Kalachanis & Psaras, 2007), which commonly is affected by external factors and environmental conditions, also impacts formation of lenticels (Kuo-Huang & Hung, 1995). Any changes in stem lenticel density may influence development of stem and root aerenchyma tissues, and thus potentially alter rates of $CH_4$ transport.

### *3.5.3. Mechanisms of $CH_4$ transport through* Alnus glutinosa

Leaf physiological factors did not display a strong relationship with stem and whole-mesocosm $CH_4$ emissions between 09:00 and 16:00 h (fortnightly measurement); however during the diurnal flux experiment, weak positive relationships were observed between stem-$CH_4$ emission rates and whole tree stomatal conductance and transpiration (Table 3.4). These relationships, albeit weak, indicate that leaf gas exchange may influence tree-mediated $CH_4$ emissions, a suggestion also proposed by Garnet *et al.* (2005) for $CH_4$ fluxes from *Taxodium distichum*. These factors may also have contributed to the transport and emission of $CH_4$ through stem surfaces via the transpiration stream as a result of lateral and radial diffusion of $CH_4$ within stems. The difference between stem and whole-mesocosm $CH_4$ emissions during day and night periods (<13%; Table 3.3) offer evidence in favour of a small contribution to $CH_4$ transport via transpiration stream in *Alnus glutinosa*, nonetheless, the observed diel variation could also be due to additional mechanisms such as changes in wind speed (enhanced venturi-induced convection or mechanical disturbance), air and soil temperature (affecting solubility of $CH_4$ and diffusion capacity) and pressurised $CH_4$ transport (Schütz *et al.,* 1991). There was no evidence of substantial pressurised $CH_4$ transport in the *Alnus glutinosa* saplings. If pressurised $CH_4$ transport was an important process, tree-mediated $CH_4$ fluxes should have decreased at night similar to reduced rates of $CH_4$ export observed in herbaceous wetland plants, such as *Phragmites australis and Typha* spp. (Chanton *et al.,* 1993; van der Nat *et al.,* 1998). Instead, a < 13% difference was observed between stem and whole-mesocosm $CH_4$ emissions during day and night periods (Table 3.3).

Results from this study suggest that $CH_4$ is transported through *Alnus glutinosa* predominantly by molecular diffusion and released from stem surfaces via lenticels. This

assertion is supported by the following observations: i) the highest rates of stem-$CH_4$ emissions were observed at the lowest sections of stem and stem-$CH_4$ emission rates decreased with increasing height on stems (Fig. 3.1); ii) there was an absence of measurable $CH_4$ egress through leaves, a lack of or weak stomatal control over stem and whole-mesocosm $CH_4$ emission rates (Tables 3.4, 3.5 and 3.6), and no distinctive diel patterns in tree-mediated $CH_4$ emissions (Fig. 3.2; Table 3.3); iii) the density of stem lenticels related positively, linearly and strongly with rates of stem-$CH_4$ flux (Fig. 3.4; Table 3.5); and iv) $CH_4$ flux strength related positively and linearly with pore-water $CH_4$ concentration (Fig. 3.3a; Tables 3.5 and 3.6). These findings are consistent with observations by Terazawa *et al.* (2007) of stem-$CH_4$ emissions from *Fraxinus mandshurica* var. *japonica* during the leafless season and the report by Garnet *et al.* (2005) of the absence of a mid-morning $CH_4$ emission maxima and a non-hysteretic $CH_4$ emission response curve for *Taxodium distichum*. Collectively these observations (this study and Garnet *et al.*, 2005; Terazawa *et al.*, 2007) provide compelling evidence for the importance of diffusive transport through stems in driving $CH_4$ transport and emission from trees.

## 3.6. Conclusions

This study provides additional evidence for the capacity of trees to mediate export of significant quantities of soil-derived $CH_4$ to the atmosphere and reinforces the need to include measurements of $CH_4$ fluxes from trees in emission inventories of forested wetlands. It specifically identifies principal mechanisms and controls on $CH_4$ flux from *Alnus glutinosa*, demonstrating that stem surfaces dominate $CH_4$ egress and that no measurable quantity of $CH_4$ is emitted from leaves. Consequently, upscaling of tree-mediated $CH_4$ emissions from forested wetlands should use the LAI proxy cautiously.

Further work is needed to characterise the capacity and mechanisms by which other flood-tolerant tree species may mediate transport of $CH_4$ from soil to the atmosphere in order to accurately quantify the role of forested wetlands in the global $CH_4$ cycle.

# CHAPTER FOUR

# Tree Stem Methane Emissions in a Temperate Forested Wetland: Controls and Ecosystem Contributions

## 4.1. Abstract


- Wetland-adapted trees are known to transport soil-produced $CH_4$, an important greenhouse gas, to the atmosphere, yet seasonal variations and controls on the magnitude of tree-mediated $CH_4$ emissions remain unknown for mature forests.

- The spatial and temporal variability in stem-$CH_4$ emissions *in situ* and their controls in two wetland-adapted tree species (*Alnus glutinosa* and *Betula pubescens*) located in a temperate forested wetland were examined. Soil and herbaceous plant-mediated $CH_4$ emissions (from hollows and hummocks) also were measured, thus enabling an estimate of contributions from each pathway to total ecosystem flux.

- Stem-$CH_4$ emissions varied significantly between the two tree species, with *Alnus glutinosa* displaying minimal seasonal and diurnal variations while substantial seasonal and diurnal variations were observed in *Betula pubescens*. Trees from each species emitted similar quantities of $CH_4$ from their stems regardless of

whether they were situated in hollows or hummocks. While soil temperature and pore-water $CH_4$ concentrations best explained annual variability in stem emissions, wood specific density and pore-water $CH_4$ concentrations best accounted for between species variations in stem-$CH_4$ emission.

- This study demonstrates that in a temperate forested wetland, tree-mediated $CH_4$ emissions contribute up to 27% of the ecosystem $CH_4$ flux, with the largest contributions occurring in spring and winter. Further studies are required to measure and fully integrate this emission pathway in other types of forested wetlands.

## 4.2. Introduction

Wetlands comprised of open waters, herbaceous vegetation and wetland-adapted trees release as much as 170 Tg $CH_4$ $a^{-1}$ (Bergamaschi *et al.,* 2007) globally, however, there is large uncertainty associated with this estimate (Dlugokencky *et al.,* 2003; Bousquet *et al.,* 2006) which has hindered efforts to accurately predict ecosystem feedbacks to climate change. Furthermore, there have been contradictory explanations for recently observed variations in atmospheric $CH_4$ concentration (Aydin *et al.,* 2011; Kai *et al.,* 2011; Simpson *et al.,* 2012), with recent reports invoking new and previously unaccounted for sources of $CH_4$ in forested wetlands (Martinson *et al.,* 2010; Bastviken *et al.,* 2011), principally in tropical and subtropical regions. An improved understanding of the magnitude and relative contributions of various wetland $CH_4$ production processes and release pathways is therefore essential in order to constrain uncertainties and accurately predict their response to future changes in climate.

Tree-mediated $CH_4$ emission is arguably one of the least studied $CH_4$ emission pathways. In contrast, herbaceous plant-mediated $CH_4$ emissions have been studied for over two decades across various ecosystems: rice paddies (e.g., Holzapfel-Pschorn & Seiler, 1986; Hosono & Nouchi, 1997; van Bodegom *et al.,* 2001), tropical wetlands (e.g., Bartlett *et al.,* 1988) and boreal peatlands (e.g., Whalen & Reeburgh, 1992; Whiting & Chanton, 1992). There is reasonable understanding of species differences, diurnal and seasonal variation and controls on these emissions (e.g., Witting & Chanton, 1990; Chanton & Dacey, 1991; Schütz *et al.,* 1991; Grünfeld & Brix, 1999). As a result, plant-mediated $CH_4$ emissions are normally well-represented in ecosystem $CH_4$ flux estimates. Similarly, a substantial body of literature also exists on diffusion and ebullition pathways, resulting in these pathways being integrated into the ecosystem flux estimate of a wide range of ecosystems (e.g., Bartlett *et al.,* 1988; Engle & Melack, 2000; Comas *et al.,* 2007; Coulthard *et al.,* 2009; Bastviken *et al.,* 2011).

Early studies by Rusch & Rennenberg (1998) using wetland-adapted saplings (*Alnus glutinosa*) revealed the existence of significant $CH_4$ emissions via stem surfaces and its relationship with $CH_4$ in the root zone. Sporadic studies since then using other tree species have consistently confirmed the presence of tree-mediated $CH_4$ emissions and identified some of the controls. However, these studies have been laboratory based, i.e., carried out using mesocosms or microcosms (e. g., Rusch & Rennenberg, 1998; Garnet *et al.,* 2005); or short-term when carried out *in situ* (Terazawa *et al.,* 2007; Gauci *et al.,* 2010) and mainly limited to temperate ecosystems. We therefore know very little about how this conclusively demonstrated but poorly quantified pathway contributes to ecosystem $CH_4$ emissions relative to other $CH_4$ transport pathways. Direct evidence of the potential influence of tree-mediated $CH_4$ emissions on wetland $CH_4$ budgets is lacking.

Spatial and seasonal variations in northern wetlands that strongly influence net $CH_4$ emissions are linked to variations in temperature, water-table depths and plant species composition and traits (Whiting & Chanton, 1992; Turetsky *et al.,* 2002; Bubier *et al.,* 2003; Christensen *et al.,* 2003; Ström *et al.,* 2003, 2005; Bloom *et al.,* 2010). However, seasonal variations in tree-mediated $CH_4$ emissions and their primary drivers are yet to be characterised in a forested wetland. The two studies of seasonal variations in stem-$CH_4$ emissions report contrasting observations (Terazawa *et al.,* 2007; Gauci *et al.,* 2010). The short observation period (only spanning part of the growing season) and relatively small sample size limits inferences that can be drawn from these two studies.

This study aimed to fully quantify seasonal variations of $CH_4$ emissions from different pathways within a temperate forested wetland, in particular, focusing on tree-mediated $CH_4$ emissions from two mature wetland-adapted tree species, *Alnus glutinosa and Betula pubescens*, which occur extensively throughout the northern hemisphere. The following hypothesis were tested in this study: i) wetland trees adapted to anoxic soils release large quantities of $CH_4$ and vary seasonally due to changes in environmental variables that regulate tree growth and soil $CH_4$ production; ii) quantities of $CH_4$ released vary between tree species due to differences in morphological adaptations; iii) soil temperature and water-table depth act as important regulators of tree-mediated $CH_4$ emissions, with emissions increasing with increasing soil temperature and water-table position.

## 4.3. Materials and methods

### *4.3.1. Site description*

Methane emissions were measured in a temperate forested wetland which is described fully in Chapter 2 (section 2.2.1).

### *4.3.2. Methane measurement*

#### *4.3.2.1. Seasonal variation*

Methane emissions from tree stems, hollows and hummocks (vegetated and non-vegetated) were measured fortnightly using a range of static chambers for a year, from April 2011 to April 2012, with the exception of January and February 2012 when monthly measurements were performed. Static chambers used to measure $CH_4$ emissions from tree stems, hollows and hummocks (non-vegetated, six each and vegetated, four each) are described in Chapter 2 (section 2.3). Methane emissions from the stems of two wetland tree species (*Alnus glutinosa* and *Betula pubescens*) with stem diameters of 7.5-19.5 cm, eight trees each, were measured at three heights: 20-50 cm, 60-90 cm and 100-130 cm above the soil surface. However, in order to investigate the emissions along the length of the tree, $CH_4$ emissions were measured at an additional stem height (140-170 cm), for two trees of each species, on each occasion. Additionally, the following two sets of experiments were performed. Methane emissions from an additional 30 trees (18 of *Betula pubescens* and 12 of *Alnus glutinosa*) with stem diameters ranging from 7-19 cm were measured at three stem heights in August, in order to assess the spatial variability of stem-$CH_4$ emissions within the plot and the controls affecting these emissions. In September, November, January and April, $CH_4$ emissions from young trees of both tree species, 8 trees each (stem diameter of 3-7 cm), were measured at 10 cm intervals between 5 and 175 cm stem height to compare

these young tree emissions with those of mature trees. As stem-$CH_4$ emissions from young trees were not measured year round, emissions measured in September, November, January and April were used as summer, autumn, winter and spring fluxes, respectively by assuming that these emissions were representative of the entire season.

In August, when stem-$CH_4$ emissions from an additional 30 trees were measured, temporary pore-water samplers were installed within 1 m radius of the trees under investigation. The sampler design and gas extraction method are described in Chapter 2 (section 2.4.2) and were similar to the samplers used in the pilot study conducted in tropical forested wetland. Using these samplers, soil water was extracted between 20 and 30 cm soil depth and analysed for pore-water $CH_4$ concentrations.

*4.3.2.2. Diurnal variation*

Diurnal variations in $CH_4$ emissions from stem surfaces (four trees per species) and soil surfaces (vegetated and non-vegetated; four each), were investigated twice, a 48-hr study in mid-August (summer) and a 24-hr study in late-November (autumn), with a 4-hr sampling interval (06:00-10:00, 10:00-14:00, 14:00-18:00, 18:00-22:00, 22:00-02:00 and 02:00-06:00 hr). In August, the difference between day and night air temperature was approximately 6 °C, however, in November, the air temperature gap widened (approximately 11 °C difference) but on both occasions soil temperatures stayed relatively similar between day and night, probably due to the upwelling hydrology. Both tree species had no leaves in November during diurnal measurements. PAR was recorded during these 4-hr sampling intervals, using a quantum sensor (Skye Instruments Ltd., Powys, UK) approximately 750 m away from the forest canopy.

*4.3.3. Environmental Controls*

Two thermocouples (Type T Thermocouple, RS® components Ltd., Corby, UK) were installed at 30 cm soil depth at two locations within the plot, each with hollows and hummocks, which recorded soil temperature. The soil-water temperature at the surface also was recorded at two locations in hollows (64K HOBO Pendant Temp Logger, Tempcon Instrumentation, West Sussex, UK). Additionally, on each measurement occasion, air temperature, relative humidity and atmospheric pressure also were recorded using a hand held probe (TR-73U thermo recorder, T & D Corporations, Nagano, Japan). Within the study plot, two piezometers (2.5 cm diameter PVC pipes with 0.5 cm holes drilled at various intervals) were installed each within hollows and hummocks, and water-table levels were measured manually on each measurement occasion. Due to the upwelling hydrology, the water-table levels always stayed at the surface in the hollows (average of 3.5 cm above soil surface) and fluctuations were small in hummocks, with a maximum water-table draw down of 14.5 cm measured in the hummocks (May 2011). PAR also was recorded thrice during each measurement campaign approximately 750 m away from the forest canopy.

An increment borer was used to extract wood samples at stem heights of 35, 75, 115 and 130 cm from both the tree species (26 of *Betula pubescens* and 20 of *Alnus glutinosa*). The wood samples were collected after the flux measurements were concluded (June 2012). The specific density of the wood was calculated based upon its dry mass and volume as described in Chapter 2 (section 2.7).

*4.3.4. Statistical analysis*

All statistical analyses were performed using SPSS v.19 (SPSS, Chicago, IL, USA) with a significance level of $P \leq 0.05$. All values presented are mean ± SE. All datasets were first

tested for: i) normal distribution using a Shapiro-Wilk-test; ii) equality (homogeneity) of variances in different subpopulations using Levene's test; and iii) outliers using box-plots. Methane emissions from *Betula pubescencs* from all three measurement heights and vegetated hollows were not normally distributed. Although various transformations were attempted, these still failed to meet the criteria for normal distribution. Therefore non-parametric Kruskal-Wallis test was used to compare averages of $CH_4$ flux from each pathway for each sampling occasion followed by group comparisons using Mann-Witney U test. All diurnal $CH_4$ fluxes met the assumptions of normality. Diel variations in $CH_4$ fluxes over 48-hr period (August 2011) and 24-hr period (November 2011) were tested using ANOVA repeated measures. The relationship between diurnal $CH_4$ fluxes and environmental controls were analysed using regressions analysis. Relationships between $CH_4$ emissions from stem and soil surfaces (vegetated and non-vegetated) and independent variables were analysed using univariate regression analysis, as all assumptions of regression were met. Stepwise multiple regression analysis was used to identify the best explanatory variable. Soil temperature and air temperature were highly correlated ($R$ = 0.98) and therefore only soil temperature measured at 30 cm below the soil surface was used in multiple regression analysis. The means of stem-$CH_4$ emissions measured from an additional 30 trees in August (one-off study) were compared using a t-test and the relationships between the variables (stem diameter, wood specific density and pore-water $CH_4$ concentration) and stem-$CH_4$ emissions were analysed using regression analysis and a mixed model.

## 4.4. Results

### 4.4.1. Seasonal variation

#### *4.4.1.1 Stem-$CH_4$ emission pathway*

Both tree species released significant quantities of $CH_4$ via their stems throughout the observation period, with fluxes varying significantly over the observation period ($P < 0.001$) and between the two species (Fig. 4.1). Stem-$CH_4$ emissions did not differ significantly in trees located in hollows and hummocks ($P > 0.05$). Stem-$CH_4$ emissions measured from an additional 30 trees in August (one-off study) further supported this observation. In August, the average fluxes from *Betula pubescens* (n = 18) were 188 ± 21.4 μg $m^{-2}$ $hr^{-1}$ and 174 ± 8.64 μg $m^{-2}$ $hr^{-1}$, and from *Alnus glutinosa* (n = 12) they were 178 ± 6.3 μg $m^{-2}$ $hr^{-1}$ and 166 ± 13.8 μg $m^{-2}$ $hr^{-1}$, from the hollows and hummocks respectively. Stem-$CH_4$ fluxes measured from the additional 30 trees were no different ($P > 0.05$) from the stem-$CH_4$ fluxes measured from the eight trees, thus confirming that the eight trees studied all year round were representative of the study plot as a whole.

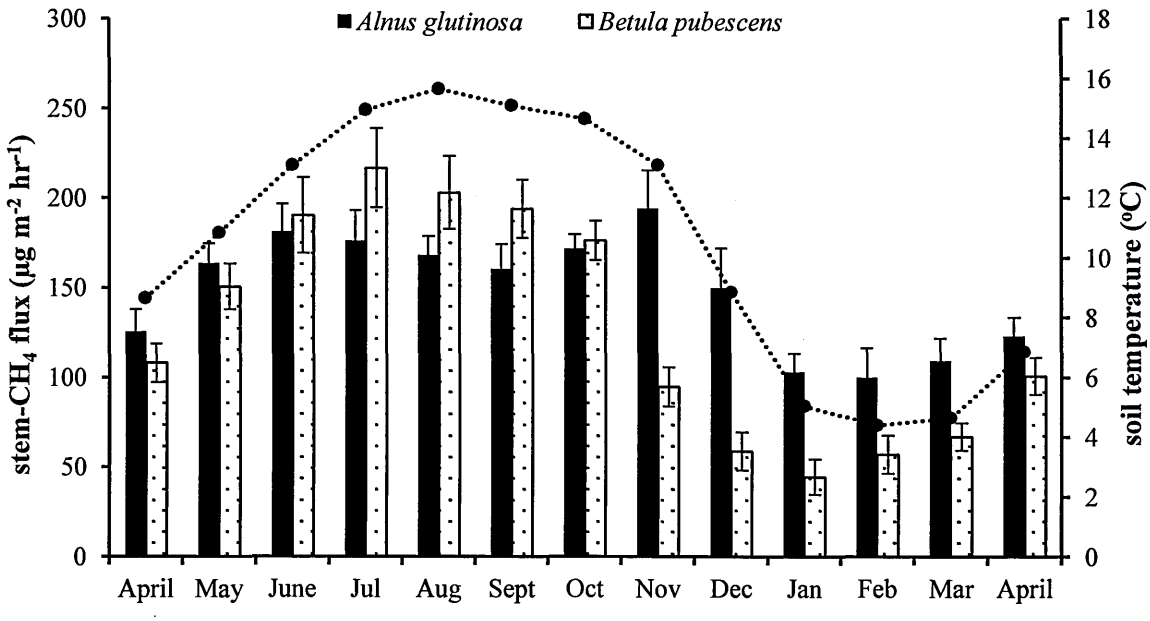


Figure 4.1: Mean stem-$CH_4$ fluxes (± SE; n = 8) from *Alnus glutinosa* and *Betula pubescens* measured at 20-50 cm stem height above the soil surface between April 2011 and April 2012.

Stem-$CH_4$ emissions varied seasonally ($P < 0.001$) and differed between the two tree species. Stem-$CH_4$ emissions from *Alnus glutinosa* increased from April to June, stayed relatively constant between July and October, increased again in November to a peak of 194 ± 21 µg $m^{-2}$ $hr^{-1}$, and then decreased from late December through to March. Although, emissions from *Betula pubescens* displayed similar patterns to *Alnus glutinosa* between April and October, its stem emissions decreased in November and then remained relatively constant until March (Fig. 4.1). In general, stem-$CH_4$ emissions were lower in winter from both the tree species. The highest stem flux of 194 ± 21 and 216 ± 22 µg $m^{-2}$ $hr^{-1}$ from *Alnus glutinosa* and *Betula pubescens,* respectively, occurred in November and July (Fig. 4.1).

Stem-$CH_4$ emissions from *Betula pubescens* were significantly higher in the summer (June-August) than from *Alnus glutinosa*, while the opposite was true in autumn

(September-November) and winter (December-February). Furthermore, the seasonal pattern in stem emissions from *Betula pubescens* was more pronounced than that of *Alnus glutinosa*. For example, the $CH_4$ emissions from *Alnus glutinosa* were 175 ± 14 μg $m^{-2}$ $hr^{-1}$ in summer, 48.3% more than the emission rate in winter (118 ± 16 μg$m^{-2}$ $hr^{-1}$). However, summer fluxes for *Betula pubescens* were 3.8 times more than winter fluxes, with summer and winter emission rates being 203 ± 21 μg $m^{-2}$ $hr^{-1}$ and 53.5 ± 10 μg $m^{-2}$ $hr^{-1}$, respectively.

Stem-$CH_4$ emissions within and between the two tree species were highly variable. In general, stem-$CH_4$ emissions decreased with stem height in both the tree species. However, the relationship between stem emissions and stem height varied for *Betula pubescens* throughout the observation period. A power function relationship between stem sampling height and stem flux was observed between April and October in both the tree species. Between November and March, stem emissions were linearly related to stem sampling height in *Betula pubescens*, while *Alnus glutinosa* displayed a power function relationship (Table 4.1). Stem-$CH_4$ emissions measured at the fourth stem sampling height (140-170 cm above the soil surface) were consistent with relationships observed between stem sampling height and stem fluxes from measurements made at lower sampling heights in both tree species.

Table 4.1: Relationship between stem-$CH_4$ fluxes from mature trees and stem sampling height above the wetland forest floor (20-50 cm, 60-90 cm and 100-130 cm above the soil surface) for the two tree species studied.

| | Relationship ($R^2$) | |
|---|---|---|
| | *Alnus glutinosa* | *Betula pubescens* |
| April 2011 | $y = 402(x^{-0.322})$ (0.964) | $y = 678(x^{-0.511})$ (0.991) |
| May 2011 | $y = 715(x^{-0.403})$ (0.949) | $y = 1270x^{-0.588}$) (0.973) |
| June 2011 | $y = 527(x^{-0.297})$ (0.985) | $y = 1146(x^{-0.499})$ (0.984) |
| July 2011 | $y = 797(x^{-0.413})$ (0.944) | $y = 1349(x^{-0.5083})$ (0.980) |
| August 2011 | $y = 472(x^{-0.289})$ (0.997) | $y = 1531(x^{-0.558})$ (0.970) |
| September 2011 | $y = 585(x^{-0.355})$ (0.926) | $y = 1297(x^{-0.525})$ (0.936) |
| October 2011 | $y = 790(x^{-0.422})$ (0.955) | $y = 1240(x^{-0.540})$ (0.978) |
| November 2011 | $y = 575(x^{-0.302})$ (0.981) | $y = -0.55x + 110$ (0.981) |
| December 2011 | $y = 449(x^{-0.292})$ (0.988) | $y = -0.398x + 71.1$ (0.990) |
| January 2012 | $y = 278(x^{-0.279})$ (0.999) | $y = -0.274x + 52.1$ (0.977) |
| February, 2012 | $y = 360(x^{-0.352})$ (0.953) | $y = -0.401x + 69.8$ (0.991) |
| March 2012 | $y = 331(x^{-0.308})$ (0.983) | $y = -0.393x + 77.1$ (0.965) |
| April 2012 | $y = 348(x^{-0.291})$ (0.994) | $y = 3151(x^{-0.95})$ (0.949) |

y = average stem-$CH_4$ flux ($\mu g\ m^{-2}\ hr^{-1}$) for each 30 cm section of the tree that was measured; x = average stem height (cm) of that 30 cm section.

Methane fluxes from young *Alnus glutinosa* and *Betula pubescens* were significantly greater than mature trees (Fig. 4.2) at all measurement occasions, although the magnitude varied between the two tree species (Fig. 4.2). In September, young *Alnus glutinosa* released 2242 ± 347 $\mu g\ m^{-2}\ hr^{-1}$ from 5 to 35 cm stem height compared with 160 ± 14 $\mu g\ m^{-2}\ hr^{-1}$ from 20 to 50 cm stem height, *c.* 14 times more $CH_4$ than the mature trees. Similarly, young *Betula pubescens* released *c.* 6.5 times more $CH_4$ than the mature trees, averaging 1248 ± 228 $\mu g\ m^{-2}\ hr^{-1}$ and 194 ± 16 $\mu g\ m^{-2}\ hr^{-1}$, respectively. The differences in

magnitude between mature and young stem-$CH_4$ fluxes decreased for *Betula pubescens* in November and January but stayed relatively constant for *Alnus glutinosa* in the same period. Methane also was released along the length of the tree from all of the young trees but displayed a linear relationship with stem height (Table 4.2) rather than a power function relationship in mature trees.

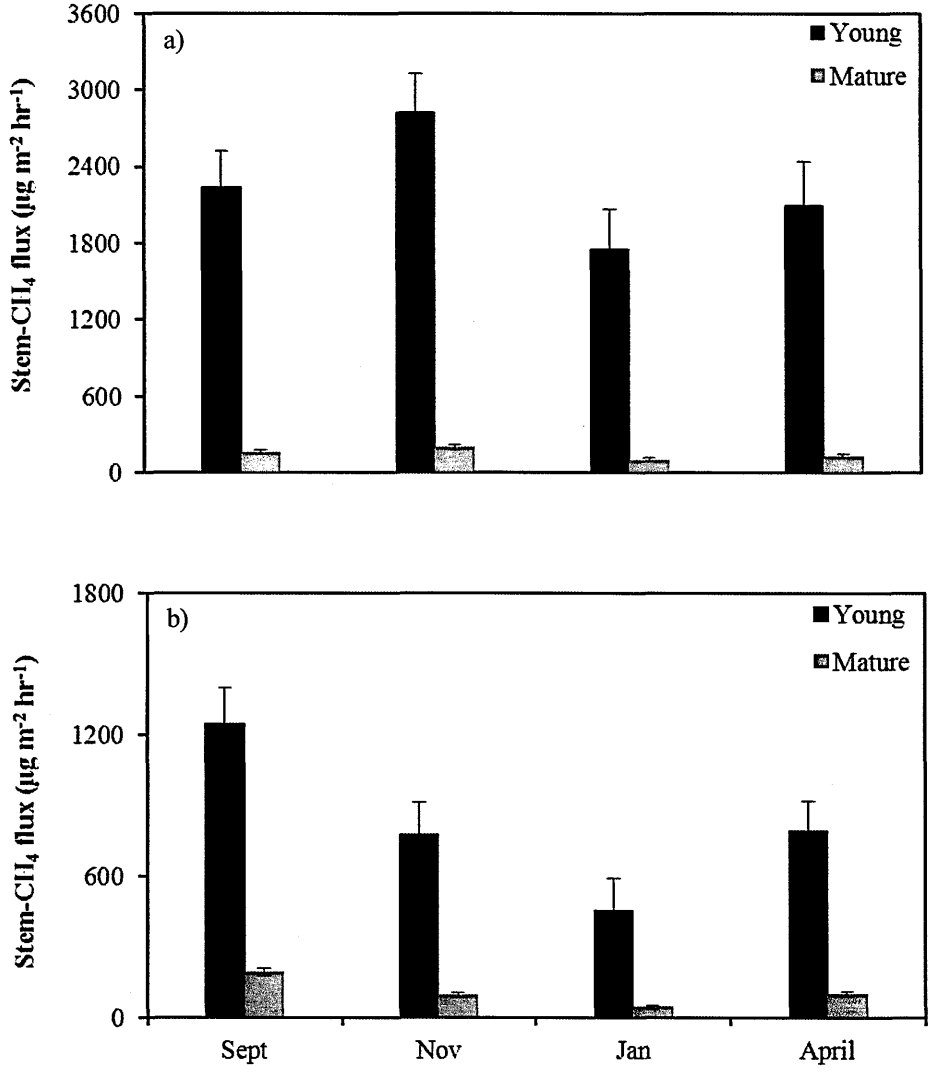


Figure 4.2: Mean stem-$CH_4$ fluxes (± SE; n = 8) from young and mature a) *Alnus glutinosa* and b) *Betula pubescens* measured at 5-35 cm and 20-50 cm stem height above the soil surface, for young and mature trees, respectively.

Table 4.2: Relationship between stem-$CH_4$ fluxes from young trees and stem sampling height above the wetland forest floor (5-35 cm, 40-70 cm, 75-105 cm, 110-140 cm and 145-175 cm above the soil surface) for the two tree species studied.

| | Relationship ($R^2$) | |
|---|---|---|
| | *Alnus glutinosa* | *Betula pubescens* |
| September 2011 | y = -15.4x + 2550 (0.964) | y = -7.92x + 1356 (0.966) |
| November 2011 | y = -19.3x + 3271 (0.978) | y = -5.71x + 894 (0.95) |
| January 2012 | y = -10.9x + 1839 (0.943) | y = -2.72x + 465 (0.933) |
| April 2012 | y = -13.8x + 2353 (0.934) | y = -4.85x + 849 (0.978) |

y = average stem flux ($\mu g\ m^{-2}\ hr^{-1}$) for each 30 cm section of the tree that was measured; x = average stem height (cm) of that 30 cm section.

*4.4.1.2. Non-tree $CH_4$ emission pathways*

Vegetated soil surfaces (hollows and hummocks) released significantly more $CH_4$ than non-vegetated soil surfaces during the growing season (Fig. 4.3). Methane emissions from hollows (vegetated and non-vegetated) and hummocks (non-vegetated) showed a typical seasonal pattern during the measurement period (Fig. 4.3), with the exception of an additional peak observed in hollows (non-vegetated) in November soon after autumnal leaf loss. Methane emissions from hollows (vegetated and non-vegetated) reached their maximum in summer (June-September) when the water and soil temperatures were highest. As the soil and water temperatures dropped, $CH_4$ emissions from hollows declined and were negligible when the soil temperature was < 5 °C (December-February). Methane emissions from vegetated hummocks and hollows ranged from negligible emissions in winter to a maximum of 524 ± 74 $\mu g\ m^{-2}\ hr^{-1}$ and 774 ± 67 $\mu g\ m^{-2}\ hr^{-1}$, respectively, in summer. Although, $CH_4$ emissions from hummocks followed a similar pattern, emissions were more variable due to their response to water-table fluctuations. Methane emissions

from vegetated hummocks were influenced by water-table fluctuations and mimicked the pattern displayed by $CH_4$ emissions from hollows. Methane fluxes from vegetated soil surfaces (hollows and hummocks) were higher than stem-$CH_4$ fluxes from May to November but were significantly smaller in winter (December-February).

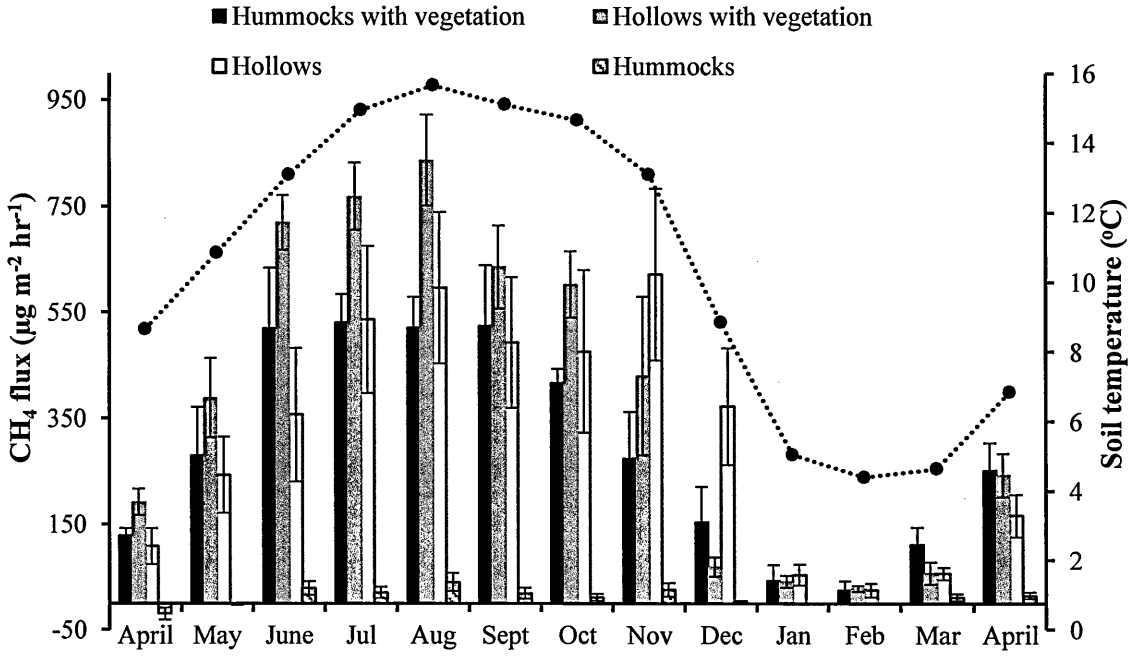


Figure 4.3: Mean $CH_4$ emissions (± SE) measured from hollows (non-vegetated; n = 6), hummocks (non-vegetated; n = 6), hollows (vegetated; n = 4) and hummocks (vegetated; n = 4).

### *4.4.2. Diurnal variations*

#### *4.4.2.1. Stem-$CH_4$ emission pathway*

Diurnal variations in stem-$CH_4$ fluxes significantly varied between sampling occasions ($P < 0.01$) and the two tree species ($P < 0.01$; Fig. 4.4). Stem-$CH_4$ fluxes from *Betula pubescens* showed a typical diurnal pattern in summer but such pattern was less prominent in autumn. On both occasions (summer and autumn), diurnal patterns in stem-$CH_4$ fluxes

from *Alnus glutinosa* were not apparent. In summer, $CH_4$ fluxes from *Betula pubescens* increased and decreased with corresponding light levels but no distinct peaks (e.g., early morning or noon peaks) were evident. The day time stem-$CH_4$ emissions from *Betula pubescens* were 36.4% greater than at night. In contrast, *Alnus glutinosa* showed no marked diurnal variations in stem-$CH_4$ emissions, with day and night time emissions averaging 165 ± 13 and 142 ± 16 μg $m^{-2}$ $h^{-1}$, respectively, resulting in a 13.8% difference.

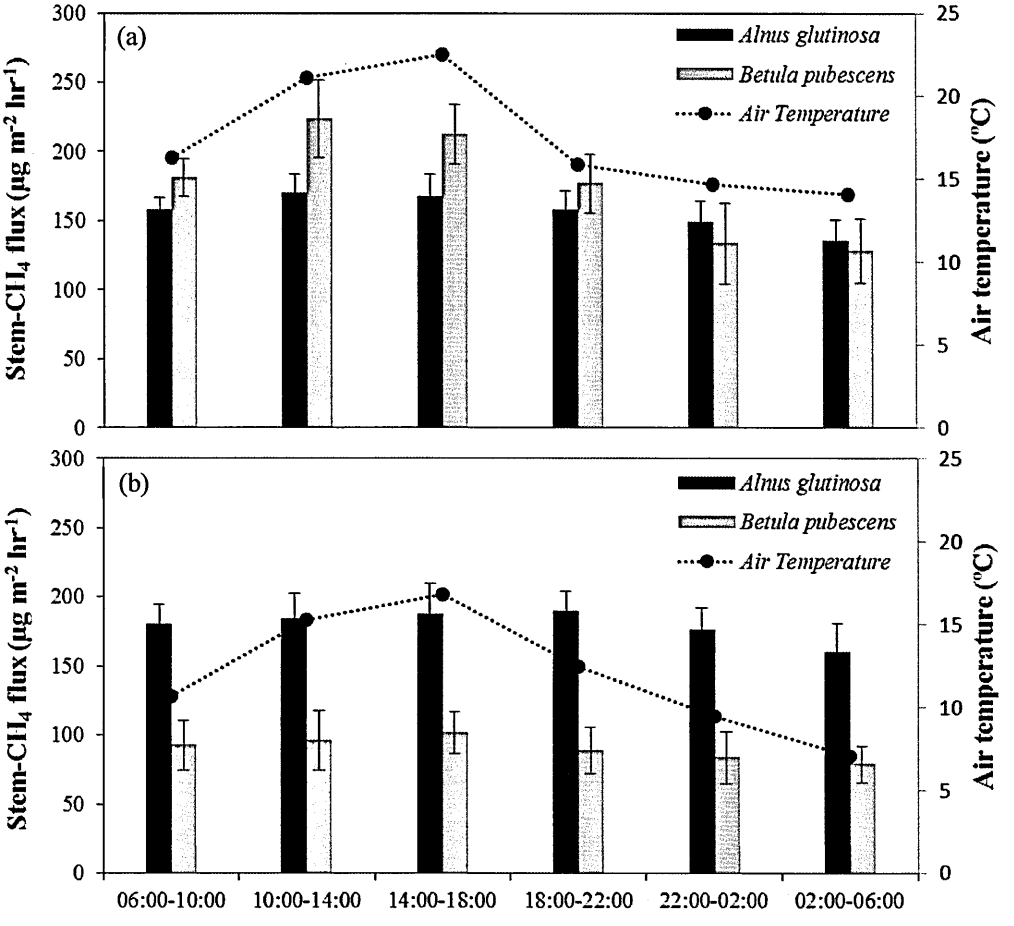


Figure 4.4: Diurnal variations in stem-$CH_4$ fluxes (± SE; n = 4) from *Alnus glutinosa* and *Betula pubescens* measured at 20-50 cm stem height above the soil surface observed in a) summer (mid-August 2011) and b) autumn (late-November 2011) over a 24-hr period.

During the day, the stem-$CH_4$ fluxes from *Alnus glutinosa* were significantly lower than that of *Betula pubescens* but the emissions from the two tree species were of similar

magnitude at night (Fig. 4.4a). Increase in stem-$CH_4$ emissions from *Betula pubescens* coincided with increasing air temperature and PAR but these factors appeared to have little effect on stem-$CH_4$ emissions from *Alnus glutinosa*.

In contrast, in autumn, stem-$CH_4$ emissions from both the tree species displayed minimal diurnal variation (Fig. 4.4b). The stem-$CH_4$ emissions during the day and night were 97.8 ± 18.1 μg $m^{-2}$ $h^{-1}$ and 81.4 ± 16 μg $m^{-2}$ $h^{-1}$ for *Betula pubescens* and 184 ± 18.2 and 168 ± 14.5 for *Alnus glutinosa*, resulting in a 16% and 8.5% difference, respectively. The distinct diurnal variation observed in summer for *Betula pubescens* was less pronounced in autumn. Stem-$CH_4$ emissions from *Alnus glutinosa* were significantly higher than emissions from *Betula pubescens* both at night and daytime. Increase in both air temperature and PAR appeared to have little effect on stem-$CH_4$ emissions from both the tree species in autumn.

*4.4.2.2. Non-tree $CH_4$ emission pathways*

In summer, diurnal patterns in $CH_4$ fluxes from vegetated soil surfaces were more apparent than non-vegetated surfaces (Fig. 4.5a). As a result, the difference between day and night time emissions for vegetated hollows and hummocks were 50% and 39%, respectively, whereas these differences were 7% and 11.5% for non-vegetated hollows and hummocks, respectively. Methane emissions from vegetated soil surfaces exceeded that of non-vegetated surfaces during the day; however, emissions from hollows (non-vegetated) were the largest at night-time.

While the diurnal variation in $CH_4$ fluxes from non-vegetated surfaces stayed relatively consistent in summer and autumn (11.5% vs. 9.1% for hummocks and 6.9% vs.7.6% for hollows in summer and autumn, respectively), the large difference between the day and

night time $CH_4$ fluxes from vegetated soil surfaces observed in summer decreased to 18% for vegetated hollows and 14.3% for vegetated hummocks in autumn, similar to diurnal patterns observed in stem-$CH_4$ emission from *Betula pubescens*. Methane emissions from non-vegetated hollows dominated day and night time emissions in autumn.

On both these occasions, soil temperature appeared to have little effect on diurnal patterns in $CH_4$ emissions from soil surfaces (vegetated and non-vegetated). However, similar to stem-$CH_4$ emissions from *Betula pubescens*, PAR and air temperature appeared to control diurnal patterns in $CH_4$ emissions from vegetated soil surfaces in summer but had little effect on $CH_4$ emissions in autumn. Non-vegetated soil surfaces demonstrated no strong relationship with increasing or decreasing light levels or air temperature on both these occasions.

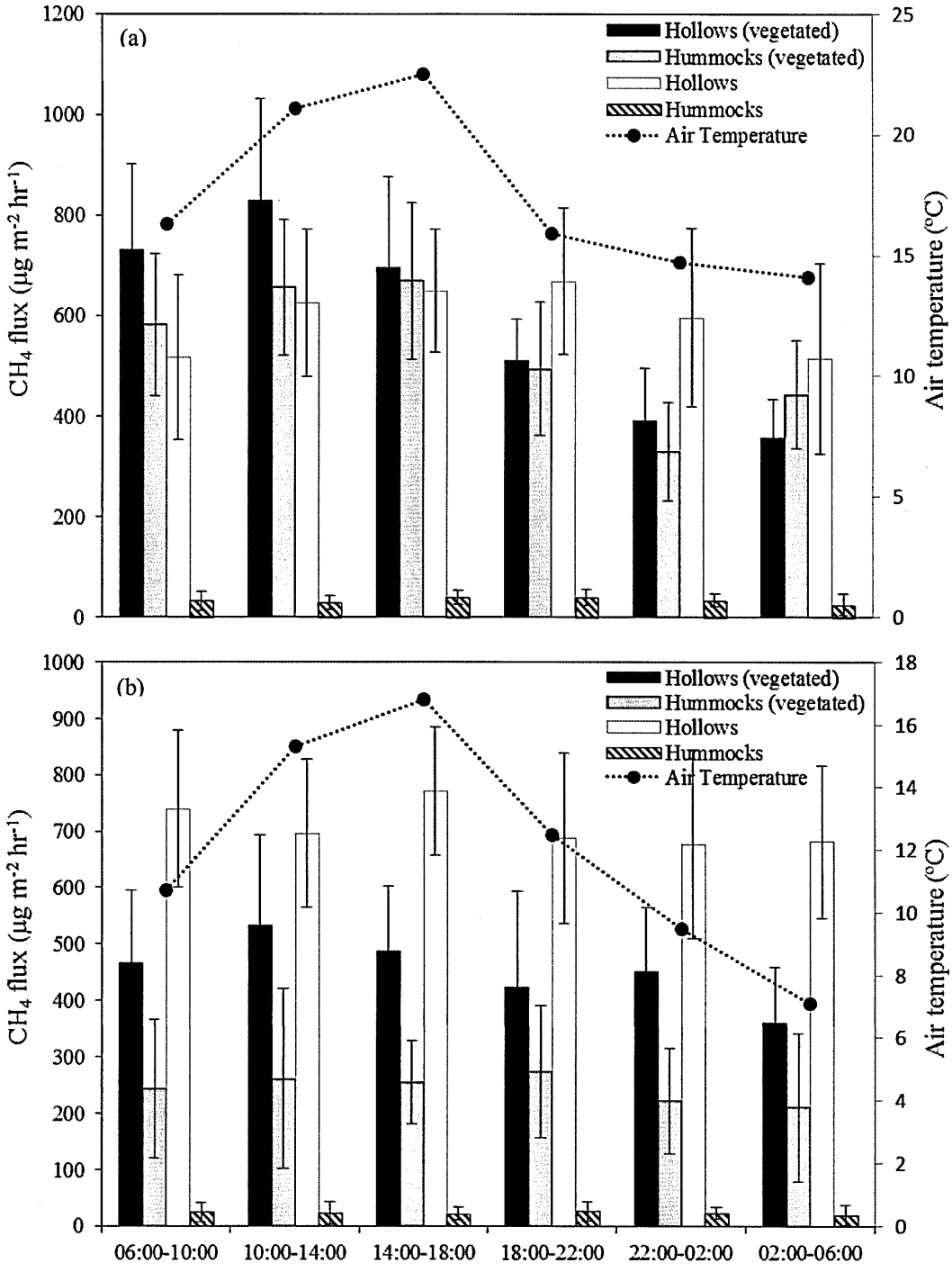


Figure 4.5: Diurnal variations in $CH_4$ fluxes (± SE; n = 4) from hollows and hummocks (vegetated and non-vegetated) measured in a) summer (mid-August 2011) and b) autumn (late-November 2011) over a 24-hr period.

*4.4.3. Ecosystem contributions*

Ecosystem contributions (fluxes per plot and percentage contributions) of the hollows (non-vegetated) and hummocks (vegetated) were highest because of their high flux rates and large $CH_4$ emitting soil surface area (Table 4.3). Contributions of tree-mediated $CH_4$ emissions (considering only the lower-most 3 m of tree emissions) varied from 5.73 ± 0.59 g $ha^{-1}$ $d^{-1}$ in summer to 2.08 ± 0.31 g $ha^{-1}$ $d^{-1}$ in winter. However, when the average tree height of ~10 m was considered, these emission estimates increased to 10.8 ± 1.1 g $ha^{-1}$ $d^{-1}$ in summer and 4.23 ± 0.58 g $ha^{-1}$ $d^{-1}$ in winter. Inclusion of young tree $CH_4$ emissions increased the above estimates to 13.2 ± 1.34 g $ha^{-1}$ $d^{-1}$ in summer and 5.65 ± 0.9 g $ha^{-1}$ $d^{-1}$ in winter (Table 4.3). Ecosystem contributions of all $CH_4$ emission pathways measured in this study varied with season, while contributions of herbaceous plant-mediated $CH_4$ emissions (vegetated hollows and hummocks) decreased from summer to winter. In contrast, tree-mediated $CH_4$ emissions displayed the opposite trend, increasing from summer to winter (i.e., 6.4-11.4% in summer and 11-20% in winter and 8.8-13.5% in summer and 17-25% in winter when emissions from young trees are considered), with highest contributions observed during spring (11-27%; Table 4.3). Notably, summer $CH_4$ emissions made up the bulk of the annual $CH_4$ emissions (*c.* 40.7%), whereas winter emissions only represented 9%.

Table 4.3: Estimated ecosystem contributions (flux per plot and percentage contributions) of $CH_4$ emissions from *Alnus glutinosa, Betula pubescens*, hollows and hummocks (vegetated and non-vegetated). The percentage contribution range for hollows and hummocks (vegetated and non-vegetated) represents the individual contributions when 3 m and 10 m of the stem height is considered. The percentage contributions listed under young trees represent the contributions of young and mature trees combined.

| | | Ecosystem $CH_4$ emissions (g $ha^{-1}$ $day^{-1}$) (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Alnus glutinosa* | | *Betula pubescens* | | Tree-mediated emissions | | Hollows | Hummocks | Hollows (vegetated) | Hummocks (vegetated) |
| | | 3 m | 10 m | 3 m | 10 m | 3 m | 10 m | | | | |
| Spring | Mature trees | 1.73 ± 0.16 ( 5.7) | 4.11 ± 0.39 (11.9) | 1.62 ± 0.17 (5.4) | 3.53 ± 0.36 (10.3) | 3.35 ± 0.33 (11.1) | 7.64 ± 0.75 (22.2) | 10.9 ± 3.01 (36.2 - 32) | 0.20 ± 0.87 (0.7 - 1) | 2.37 ± 0.41 (7.9 - 7) | 13.3 ± 4.36 (44.2 - 39) |
| | Young trees | 1.63 ± 0.21 (10.4) | 1.63 ± 0.21 (15.7) | 0.43 ± 0.11 (6.4) | 0.43 ± 0.11 (10.9) | 2.06 ± 0.32 (16.8) | 2.06 ± 0.32 (26.6) | 10.9 ± 3.01 (33.9 - 30) | 0.20 ± 0.87 (0.6 - 0.6) | 2.37 ± 0.41 (7.4 - 6.5) | 13.3 ± 4.36 (41.4 - 36.5) |
| Summer | Mature trees | 2.29 ± 0.20 (2.5) | 5.43 ± 0.48 (5.7) | 3.44 ± 0.39 (3.8) | 5.35 ± 0.62 (5.6) | 5.73 ± 0.59 (6.4) | 10.8 ± 1.10 (11.4) | 37.3 ± 10.2 (41.5 - 39) | 2.51 ± 2.03 (2.8 - 3) | 8.32 ± 0.81 (9.3 - 9) | 36.0 ± 6.24 (40.1 - 38) |
| | Young trees | 1.66 ± 0.14 (4.3) | 1.66 ± 0.14 (7.3) | 0.68 ± 0.1 (4.5) | 0.68 ± 0.1 (6.2) | 2.35 ± 0.24 (8.8) | 2.35 ± 0.24 (13.5) | 37.3 ± 10.2 (40.4 - 38.3) | 2.51 ± 2.03 (2.7 - 2.6) | 8.32 ± 0.81 (9 - 8.6) | 36.0 ± 6.24 (39.1 - 37) |
| Autumn | Mature trees | 2.30 ± 0.21 (2.9) | 5.46 ± 0.50 (6.4) | 2.52 ± 0.22 (3.1) | 5.25 ± 0.53 (6.1) | 4.81 ± 0.43 (6.0) | 10.7 ± 1.04 (12.5) | 39.8 ± 10 (49.8 - 46) | 1.56 ± 1.47 (1.9 - 2) | 5.96 ± 0.82 (7.5 - 7) | 27.8 ± 6.41 (34.8 - 32) |
| | Young trees | 2.18 ± 0.18 (5.4) | 2.18 ± 0.18 (8.6) | 0.43 ± 0.09 (3.6) | 0.43 ± 0.09 (6.4) | 2.62 ± 0.27 (9) | 2.62 ± 0.27 (15.1) | 39.8 ± 10 (48.2 - 45) | 1.56 ± 1.47 (1.9 - 1.8) | 5.96 ± 0.82 (7.2 - 6.7) | 27.8 ± 6.41 (33.7 - 31.5) |
| Winter | Mature trees | 1.57 ± 0.20 (8.1) | 3.73 ± 0.48 (17.6) | 0.51 ± 0.11 (2.7) | 0.51 ± 0.11 (2.4) | 2.08 ± 0.31 (10.9) | 4.23 ± 0.58 (20) | 11.3 ± 3.57 (59.3 - 53) | 0.09 ± 0.10 (0.5 - 0) | 0.49 ± 0.13 (2.6 - 2) | 5.09 ± 3.14 (26.7 - 24) |
| | Young trees | 1.18 ± 0.22 (13.2) | 1.18 ± 0.22 (21.3) | 0.23 ± 0.12 (3.6) | 0.23 ± 0.12 (3.2) | 1.42 ± 0.32 (16.8) | 1.42 ± 0.32 (24.6) | 11.3 ± 3.57 (56 - 50.7) | 0.09 ± 0.10 (0.4 - 0.4) | 0.49 ± 0.13 (2.4 - 2.1) | 5.09 ± 3.14 (24.4 - 22.2) |

*4.4.4. Environmental controls on $CH_4$ emissions*

Pore-water $CH_4$ concentrations varied significantly with soil depth and differed between the hollows and hummocks (Fig. 4.6; three months averaged). The concentrations in the hummocks were smaller than in hollows but measurable concentrations were observed at 15 to 70 cm beneath the hummock surface at all times. The concentrations between 5 and 20 cm soil depth differed with varying water-table depth. In the hollows, throughout the observation period, the highest concentrations were measured between 15 and 30 cm, and the lowest between 60 and 80 cm. Pore-water $CH_4$ concentrations between 5 and 40 cm in the hollows fluctuated throughout the season. Furthermore, the variations in pore-water $CH_4$ concentrations measured in hollows between 20 and 40 cm coincided with variations in soil temperatures. In contrast, the concentrations between 5 and 15 cm did not vary significantly with temperature but instead increased from November and remained relatively high until February (Fig. 4.6). The increase in pore-water $CH_4$ concentrations observed in November also was reflected in an increase in $CH_4$ emissions from the stem surfaces of *Alnus glutinosa* and non-vegetated hollows. Pore-water $CH_4$ concentrations measured between 20 and 25 cm soil depths in hollows accounted for up to 75%, 69%, 72% and 48% of the seasonal variations in $CH_4$ emissions from *Alnus glutinosa*, *Betula pubescens*, vegetated and non-vegetated hollows, respectively (Table 4.4). Whereas, pore-water $CH_4$ concentrations in hummocks measured at 10-20 cm and 40-50 cm soil depths largely explained variations in $CH_4$ emission from vegetated hummocks (Table 4.4).

Soil and air temperature were an important regulators of seasonal variations in $CH_4$ emissions from all pathways (Table 4.4; Appendix I-VI). The emissions from hollows (vegetated and non-vegetated), hummocks (vegetated) and stems of the two tree species varied exponentially with soil and air temperature (Table 4.4).

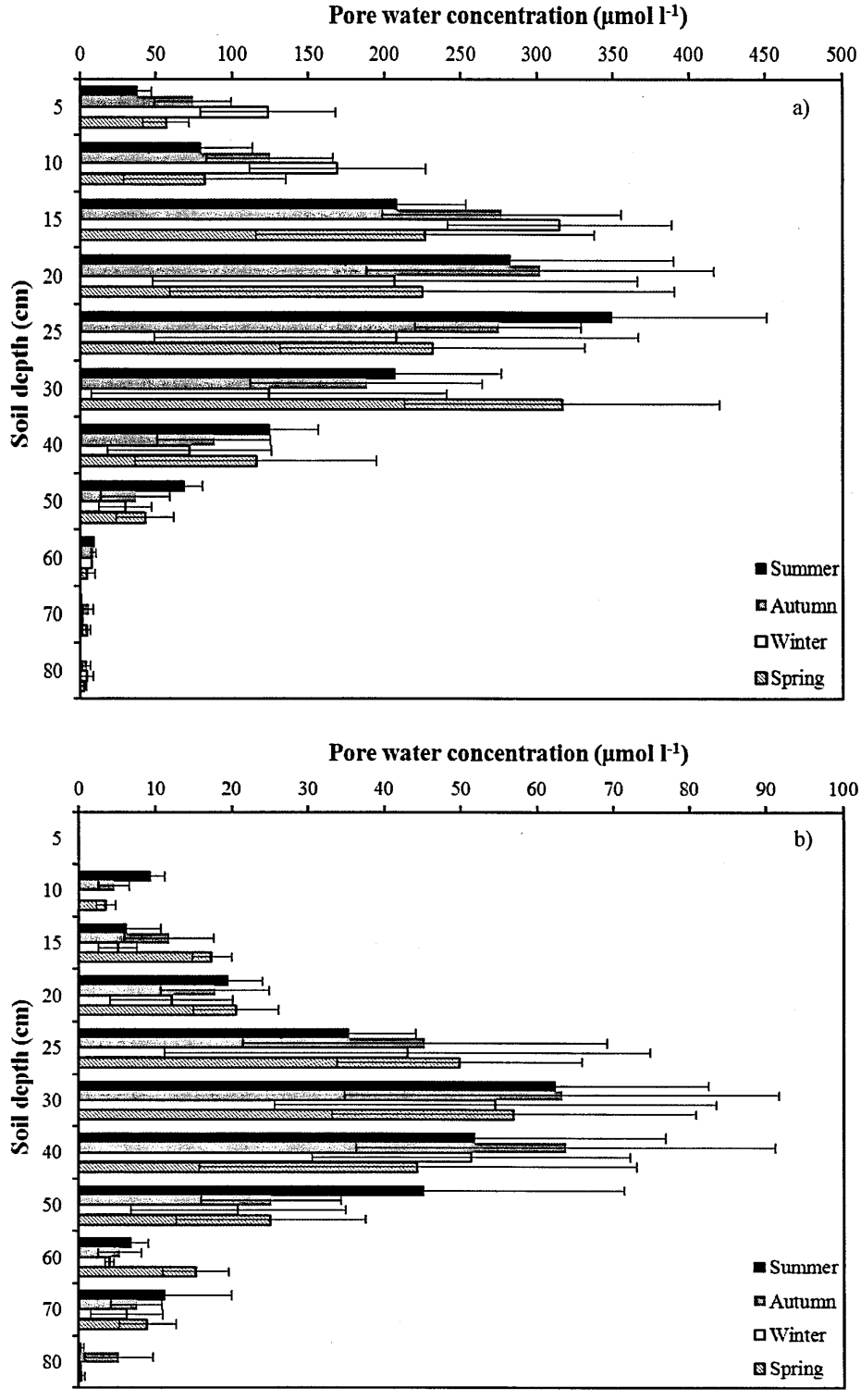


Figure 4.6: Pore-water $CH_4$ concentrations (± SE) measured at eleven soil depths (5-80 cm below the soil surface) in the a) hollows (n = 3) and b) hummocks (n = 2).

Water-table fluctuations regulated emissions from the hummocks (vegetated and non-vegetated) but played a minor role in regulating stem-$CH_4$ emissions and hollow emissions (vegetated and non-vegetated) due to the permanently high water-table depths in hollows. The results of stepwise multiple regression analysis although varied for different $CH_4$ emission pathways (Appendix I-VI), in general revealed that soil temperature and pore-water $CH_4$ concentrations explained most of the seasonal variations from all pathways, including stem-$CH_4$ emissions, while fluctuations in water-table depths only explained variations in $CH_4$ emissions from hummocks (vegetated and non-vegetated).

The wood densities at four stem height of the two tree species are listed in Table 4.5. Factors such as stem diameter, wood specific density and pore-water $CH_4$ concentrations contributed to the species' differences in stem-$CH_4$ emissions and are detailed in Table 4.6. Wood specific density appeared to increase with sampling stem height, varied between and among tree species and was statistically different between the two tree species at three sampling stem heights. However, the pore-water $CH_4$ concentrations and stem diameters were similar between the two tree species but varied within trees of the same species. Stem diameter and wood specific density were negatively related to stem-$CH_4$ flux from both tree species, the relationship being strongest at 20-50 cm stem sampling height, whereas pore-water $CH_4$ concentrations were positively related to stem-$CH_4$ fluxes (Table 4.6). While, wood specific density and pore-water $CH_4$ concentration mostly explained between species differences (appendix VII, VIII), all these variables (pore-water $CH_4$ concentration, wood specific density and stem diameter) contributed to within species variations in stem-$CH_4$ flux.

Table 4.4: Results of the relationship between the seasonal variation of the individual emission pathway (μg $m^{-2}$ $hr^{-1}$) and the controls measured in this study (slope, intercept [a, b (a = exponential relationship, b = linear relationship)]; $R^2$ value). Significant relationships are highlighted in bold.

| Measured variables | Hollows | Hummocks | Hollows vegetated | Hummocks vegetated | *A. glutinosa* | *B. pubescens* |
|---|---|---|---|---|---|---|
| Soil temperature (°C) | **(23.9, 0.213[a]; 0.814 )** | (2.09, -10.2[b]; 0.198) | **(16.9, 0.253[a]; 0.842)** | **(31.7, 0.185[a]; 0.773)** | **(89.4, 0.0471[a]; 0.762)** | **(35.7, 0.110[a]; 0.741)** |
| Air temperature (°C) | **(38.8, 0.113[a]; 0.673)** | **(1.14, -5.66[b]; 0.169)** | **(26.5, 0.143[a]; 0.779)** | **(46.2, 0.101[a]; 0.677)** | **(102, 0.023[a]; 0.537)** | **(41.4, 0.065[a]; 0.75)** |
| PAR | **(1.39, -229[b]; 0.271)** | **(0.091, -25.2[b]; 0.151)** | **(2.96, -797[b]; 0.652)** | (1.57, -330[b]; 0.466) | **(0.19, 74.8[b]; 0.239)** | **(0.605, -113[b]; 0.644)** |
| Water-table depth (cm) | - | **(5.28, 69.9[b]; 0.668)** | - | **(38.6, 728[b]; 0.385)** | (2.96, 184[b]; 0.079) | (10.5, 248[b]; 0.267) |
| $CH_4$ at 5 cm (μmol $l^{-1}$) | (-2.523, 551[b]; 0.181) | - | **(-6.25, 889[b]; 0.629)** | - | (-0.31, 172[b]; 0.132) | **(-1.45, 235[b]; 0.799)** |
| $CH_4$ at 10 cm (μmol $l^{1}$) | (-0.394, 405[b]; 0.012) | (0.472, 15.6[b]; 0.02) | **(-2.40, 697[b]; 0.252)** | **(20.1, 225[b]; 0.443)** | (-0.0011, 148[b]; 0.000005) | **(-0.675, 205[b]; 0.472)** |
| $CH_4$ at 15 cm (μmol $l^{1}$) | (-0.375, 458[b]; 0.013) | (-0.72, 23.4[b]; 0.045) | (-2.40, 1045[b]; 0.297) | **(11.4, 206[b]; 0.132)** | (-0.063, 164[b]; 0.018) | **(-0.644, 294[b]; 0.508)** |
| $CH_4$ at 20 cm (μmol $l^{1}$) | **(2.36, -229[b]; 0.483)** | **(1.67, -8.69[b]; 0.225)** | **(2.44, -196[b]; 0.293)** | **(11.4, 122[b]; 0.127)** | **(0.412, 45.7[b]; 0.747)** | **(0.396, 26.6[b]; 0.188)** |
| $CH_4$ at 25 cm (μmol $l^{-1}$) | **(1.91, -141[b]; 0.455)** | (-0.536, 40.3[b]; 0.057) | **(3.21, -425[b]; 0.722)** | (-1.31, 357[b]; 0.004) | **(0.23, 87.9[b]; 0.339)** | **(0.644, -42.5[b]; 0.688)** |
| $CH_4$ at 30 cm (μmol $l^{1}$) | (0.178, 325[b]; 0.0032) | **(0.790, -29.4[b]; 0.284)** | **(1.48, 126[b]; 0.12)** | (3.1, 117[b]; 0.052) | (0.063, 136[b]; 0.0195) | (0.335, 60.9[b]; 0.146) |
| $CH_4$ at 40 cm (μmol $l^{1}$) | (1.257, 239[b]; 0.064) | (-0.279, 32.2[b]; 0.0292) | **(3.49, 79.9[b]; 0.277)** | **(6.67, -54.1[b]; 0.198)** | **(0.279, 122[b]; 0.158)** | **(0.653, 63.3[b]; 0.23)** |
| $CH_4$ at 50 cm (μmol $l^{1}$) | (4.09, 178[b]; 0.10) | **(0.419, 5.06[b]; 0.139)** | **(13.1, -166[b]; 0.565)** | **(4.64, 164[b]; 0.203)** | **(0.779, 113[b]; 0.181)** | **(2.69, 5.89[b]; 0.571)** |
| $CH_4$ at 60 cm (μmol $l^{1}$) | (6.79, 304[b]; 0.029) | (0.634, 12.8[b]; 0.0305) | **(7.32, 354[b]; 0.019)** | (2.21, 285[b]; 0.0045) | (0.537, 144[b]; 0.009) | (2.35, 107[b]; 0.047) |
| $CH_4$ at 70 cm (μmol $l^{1}$) | (-6.44, 378[b]; 0.010) | (0.227, 15.5[b]; 0.0027) | (-16.8, 460[b]; 0.04) | (4.49, 263[b]; 0.012) | (-0.337, 149[b]; 0.0014) | (-0.961, 128[b]; 0.0031) |
| $CH_4$ at 80 cm (μmol $l^{1}$) | (-22.3, 426[b]; 0.17) | (-1.20, 19.4[b]; 0.299) | (-28.5, 498[b]; 0.155) | (26.2, 258[b]; 0.171) | **(-3.72, 159[b]; 0.237)** | (-2.92, 134[b]; 0.038) |

Table 4.5: Wood specific density (g $cm^{-3}$) measured at four stem heights for *Alnus glutinosa* and *Betula pubescens*.

| Stem height (cm) | *Alnus glutinosa* | *Betula pubescens* |
|---|---|---|
| 35 | 0.495 ± 0.023 | 0.645 ± 0.021 |
| 75 | 0.506 ± 0.015 | 0.671 ± 0.019 |
| 115 | 0.520 ± 0.027 | 0.680 ± 0.025 |
| 130 | 0.527 ± 0.019 | 0.691 ± 0.028 |

Table 4.6: The relationship between stem-$CH_4$ fluxes ($\mu g\ m^{-2}\ hr^{-1}$), stem diameter, wood specific density and pore-water $CH_4$ concentrations at 20 to 30 cm soil depth measured within 1 m radius of the trees under investigation.

| Sampling stem height | Variables measured | *Alnus glutinosa* | | *Betula pubescens* | |
|---|---|---|---|---|---|
| | | Range | Relationship ($R^2$) | Range | Relationship ($R^2$) |
| 20-50 cm | Stem diameter (cm) | 13.5 ± 0.83 | y = -5.12x + 236 (0.392)* | 12.4 ± 0.54 | y = -10.8x + 330 (0.315) * |
| | Wood specific density (g $cm^{-3}$) | 0.495 ± 0.023 | y = -238x + 288 (0.704) *** | 0.645 ± 0.021 | y = -325x + 409 (0.52) *** |
| | $CH_4$ concentration at 20-30 cm ($\mu mol\ l^{-1}$) | 237 ± 24 | y = 0.218x + 115 (0.59) *** | 272 ± 17 | y = 0.449x + 74.5 (0.536) *** |
| 60-90 cm | Stem diameter (cm) | 12.4 ± 0.76 | y = -4.43x + 191 (0.311)* | 12.1 ± 0.65 | y = -4.73x + 198 (0.165) * |
| | Wood specific density (g $cm^{-3}$) | 0.506 ± 0.015 | y = -232x + 282 (0.559) *** | 0.671 ± 0.019 | y = -230x + 297 (0.363) *** |
| | $CH_4$ concentration at 20-30 cm ($\mu mol\ l^{-1}$) | 237 ± 24 | y = 0.218x + 115 (0.59) *** | 272 ± 17 | y = 0.326x + 51.7 (0.555) *** |
| 100-130 cm | Stem diameter (cm) | 12.2 ± 0.73 | y = -2.38x + 148 (0.128)* | 11.6 ± 0.60 | y = -4.53x + 163 (0.212) * |
| | Wood specific density (g $cm^{-3}$) | 0.520 ± 0.027 | y = -119x + 182 (0.379) *** | 0.680 ± 0.025 | y = -166x + 224 (0.42) ** |
| | $CH_4$ concentration at 20-30 cm ($\mu mol\ l^{-1}$) | 237 ± 24 | y = 0.123x + 90.9 (0.38) *** | 272 ± 17 | y = 0.263x + 39.5 (0.57) *** |

*, $P < 0.05\%$; **, $P < 0.01\%$; ***, $P < 0.001\%$.

## 4.5. Discussion

This study demonstrates that tree-mediated $CH_4$ emissions contribute significantly to ecosystem $CH_4$ flux (6-22% and 8.8-27%; excluding and including young tree $CH_4$ emissions; Table 4.3) and that the largest contribution from trees occurs in spring and winter (Table 4.3), despite trees occupying less than 2% of the soil surface area. I am aware of no other study to date that reports the significance of tree-mediated $CH_4$ emissions and their contributions at an ecosystem scale.

The stems of the two tree species studied emitted significant quantities of $CH_4$ throughout the year but the magnitude and pattern of the emissions differed between the tree species and were partly independent of changes in air and soil temperature. Wetland vegetation has long been known to influence $CH_4$ emissions by altering its production, consumption and transport (Whiting & Chanton, 1992; Joabsson *et al.,* 1999; Christensen *et al.,* 2003; Ström *et al.,* 2003); however, literature on the influence of wetland-adapted trees has only emerged in the last decade. This study supports and adds to this growing literature (Rusch & Rennenberg, 1998; Vann & Megonigal, 2003; Garnet *et al.,* 2005; Terazawa *et al.,* 2007; Gauci *et al.,* 2010; Rice *et al.,* 2010) but most importantly for the first time provides insight into their ecosystem contributions over a full annual cycle by integrating all $CH_4$ emission pathways. Wetland-adapted trees, due to the formation of lenticels, aerenchyma and adventitious roots in response to flooding (Kozlowski, 1997) offer preferential pathways for the transport and release of soil produced gases such as $CH_4$ and $N_2O$ from the point of production to the atmosphere (e.g., Kozlowski, 1997; Rush & Rennenberg, 1998; Vann & Megonigal, 2003; McBain *et al.,* 2004).

Emissions of $CH_4$ through herbaceous plants (i.e., vegetated hollows and hummocks) was the largest contributor of $CH_4$ to the atmosphere (Table 4.3) during the growing season at

the study site, an observation consistent with a range of other studies that have reported that $CH_4$ transport through herbaceous plants dominates ecosystem $CH_4$ flux (e.g., Chanton & Dacey, 1991; Schütz *et al.,* 1991; Grünfeld & Brix, 1999; Greenup *et al.,* 2000). These results are not surprising given the direct and indirect effects of *Phragmites australis* and *Carex spp.* – the two species that dominate the soil surfaces at Flitwick Moor temperate forested wetland, on $CH_4$ emissions (Morrissey *et al.,* 1993; Ding *et al.,* 2003, 2005; Bergström *et al.,* 2007). Herbaceous vegetation covered 35% of the soil surface within the study plot as opposed to tree stems that covered *c.* 2% of the soil surface area. While the large area covered by herbaceous vegetation may have partly influenced their ecosystem $CH_4$ contributions (Alm *et al.,* 1999; Hirota *et al.,* 2004; Duan *et al.,* 2005; Bergström *et al.,* 2007), other studies suggest that plant species composition and traits, including the transport of $CH_4$ via well-developed aerenchyma and supply of substrates for $CH_4$ production (Levy *et al.,* 2011; Sutton-Grier & Megonigal, 2011), play a major role in controlling the magnitude and ecosystem $CH_4$ contributions. Notably, summer and spring $CH_4$ emissions were dominated by herbaceous plant-mediated transport (> 48%), however, $CH_4$ emissions from non-vegetated hollows dominated autumn and winter emissions. The shift in ecosystem contributions may have been due to autumnal vegetation senescence leading to reduction in herbaceous plant-mediated $CH_4$ transport (van der Nat & Middelburg, 1998).

The seasonal variations in stem-$CH_4$ emissions from the two tree species studied generally were similar to emission characteristics for soil surfaces both observed at Flitwick Moor temperate forested wetland and other published temperate wetland studies: high emissions in the summer and low but measurable emissions in winter (e.g., Dise *et al.,* 1993; Shannon & White, 1994; Nykänen *et al.,* 1998; Alm *et al.,* 1999; Kankaala *et al.,* 2005). Stem-$CH_4$ emissions appear to be significantly regulated by temperature (Table 4.4)

because temperature should influence both $CH_4$ production (Hosono & Nouchi, 1997) and plant productivity (Chen *et al.,* 2008). This assertion is supported by i) a strong positive relationship observed between stem-$CH_4$ emissions and temperature, $CH_4$ dissolved in pore-water between 20 and 25 cm soil depths (suggesting that warmer soil temperatures lead to increased $CH_4$ production and release; Whalen, 2005 and references within); and ii) enhanced $CH_4$ emissions observed from the two tree species from spring through to early summer during the rapid growth phase (Chen *et al.,* 2008); and iii) decreased emissions during the dormant season (Fig. 4.1).

Methane emission rate from wetlands commonly are reported to be influenced by water-table depth (e.g., Hogg *et al.,* 1992; Moore & Roulet, 1993; Waddington *et al.,* 1996; Elberling *et al.,* 2011). As a result, pore-water $CH_4$ concentrations measured in hummocks were smaller than in hollows, and appeared to affect $CH_4$ emission at the soil surface (vegetated and non-vegetated hummocks), suggesting the presence of $CH_4$ oxidation. However, water-table fluctuation did not appear to affect the magnitude of stem-$CH_4$ emissions. The upwelling hydrology of the site could explain the relatively high concentrations of $CH_4$ in the hummocks between 30 and 40 cm soil depth (Fig. 4.6), which may have supported the $CH_4$ emissions from trees in the hummocks. The presence of extensive root networks (both lateral and vertical) reaching the $CH_4$ productive zone or intercepting upwardly diffusing $CH_4$ is another plausible explanation. It is well established that the magnitude of plant-mediated $CH_4$ emissions under varying water-table is dependent on the plant rooting depth (Waddington *et al.,* 1996). However, the absence of a difference between the stem-$CH_4$ flux measured from trees in hollows and hummocks, despite the higher pore-water $CH_4$ concentrations measured in hollows between 20 and 40 cm soil depth than hummocks (Fig. 4.6), suggests that tree rooting depth and networks alone are insufficient to explain our observations.

Environmental conditions experienced by the two tree species were similar but the two species studied displayed differences in the rates and patterns of $CH_4$ flux, suggesting that variables other than temperature influence fluxes. For example, in *Alnus glutinosa*, the seasonal variation was less pronounced and an additional $CH_4$ peak was observed in autumn after leaf loss when the temperature was relatively low. However, no such peak was observed in *Betula pubescens* and emissions decreased immediately after leaf loss (Fig. 4.1). Patterns of diurnal variation also varied between the two species, i.e., the two tree species displayed contrasting diurnal variation in summer but the patterns were nearly similar in November (Fig. 4.4). The relationship between the stem height and stem-$CH_4$ emissions also varied between the tree species (linear vs. power relationships; Table 4.1 and 4.2). These differences in stem-$CH_4$ emissions could result from a number of factors that are known to influence both pre- and post-production of $CH_4$ (Sutton-Grier & Megonigal, 2011), involving complex above and below-ground interactions. Four possible reasons are proposed for the differences in stem-$CH_4$ emission characteristics between the two tree species investigated in this study.

First, different $CH_4$ transport mechanisms (passive diffusion vs. convective/transpiration driven transport) employed by the plant species influence the magnitude of plant-mediated $CH_4$ emissions (Whiting & Chanton, 1996; McBain *et al.,* 2004; Pihlatie *et al.,* 2005; Sutton-Grier & Megonigal, 2011). Species-specific differences in modes of $CH_4$ transport are well documented for a number of wetland plant species (e.g., Brix *et al.,* 1992, 1996; Chanton *et al.,* 1993; Chang *et al.,* 1998; Kim *et al.,* 1998; van der Nat *et al.,* 1998). Therefore, it is possible that the two tree species possess different $CH_4$ transport mechanisms or a combination of the two (passive diffusion and convective transport). No distinct diurnal pattern in stem-$CH_4$ emissions were observed from four-year-old *Alnus glutinosa* (Chapter 3), suggesting that the gas transport was driven mainly by passive

diffusion as no relationship between stem-$CH_4$ emissions and leaf physiological parameters was observed. This could possibly explain the observed lack of decrease in stem-$CH_4$ emission from *Alnus glutinosa* after leaf loss and minimal diurnal variation displayed by *Alnus glutinosa* both in August and November. The sudden decrease in emissions from *Betula pubescens* after leaf loss (Fig. 4.1) and the apparent absence of diurnal variation in November when compared to August (Fig. 4.4) offers some evidence for the presence of physiological control on gas transport, most likely convective/transpiration-driven gas transport, but further work is required to identify the principal mechanisms involved.

Second, wetland vegetation is known to attenuate $CH_4$ production in the root zone due to the release of $O_2$ that stimulates both methanotrophy (van der Nat & Middelburg, 1998; Joabsson & Christensen, 2001) and the regeneration of electron acceptors (Bouchard *et al.,* 2007; Laanbroek, 2010; Sutton-Grier & Megonigal, 2011). A number of studies report the influence of different types of vegetation on the attenuation of $CH_4$ production and emission (e.g., Reay *et al.,* 2005; Menyailo *et al.,* 2012). The small difference in pore-water $CH_4$ concentrations at 20-30 cm soil depth between the two tree species measured in August (272 ± 17 μmol $l^{-1}$ for *Betula pubescens* and 237 ± 24 μmol $l^{-1}$ for *Alnus glutinosa*) suggest a possible tree species effect but considering the limitations of this study (measurements not performed within close proximity of the trees under investigation through the observation period and no direct measurements of $CH_4$ oxidation), a tree species-specific effect on $CH_4$ oxidation cannot be confirmed.

Third, the release of a wide range of labile carbon compounds and nutrients through root exudation, root turnover and leaf litter stimulating $CH_4$ production (Joabsson *et al.,* 1999; Brix *et al.,* 2001; Ström *et al.,* 2003; Ström *et al.,* 2005; Dorodnikov *et al.,* 2011) is known to differ between the wetland vegetation. The type (e.g., organic acids, sugars, acetate,

phenolics and amino acids), quality (e.g., C/N in root exudates, root tissues and leaf litter; Sjogersten *et al.,* 2010; Sutton-Grier & Megonigal, 2011) and quantity of these substrates are also known to be species-dependent (Grayston *et al.,* 1996). Although no direct evidence of species-specific substrate quality is available from this study, an increase in stem-$CH_4$ emissions and pore-water $CH_4$ concentrations at 5-30 cm soil depth observed during autumn (Fig. 4.6) is likely due to increased substrate availability through autumnal leaf and root turnover (Miller *et al.,* 1999).

Lastly, differences in wetland vegetation architecture such as differences in their anatomical, morphological and physiological properties, can affect both $CH_4$ production (differences in $O_2$ and carbon inputs; Grünfeld & Brix, 1999; Colmer, 2003; Dinsmore *et al.,* 2009) and $CH_4$ transport (Schimel, 1995; Shannon *et al.,* 1996; Greenup *et al.,* 2000; Zhang *et al.,* 2011; Henneberg *et al.,* 2012). Species differences in the above and below ground biomass are known to be better predictors of the magnitude of $CH_4$ flux than other abiotic factors (Schimel, 1995; Greenup *et al.,* 2000; Henneberg *et al.,* 2012). Wood specific density at various stem heights varied within and between the two tree species but was on an average greater for *Betula pubescens* than *Alnus glutinosa*, nonetheless, wood specific density displayed an inverse relationship with stem-$CH_4$ emissions from both tree species at three sampling heights (Table 4.6). These observations offer a useful link between the tree species traits and stem-$CH_4$ emissions, suggesting that trees with increased pore spaces (as indicated by lower wood density) transport more $CH_4$. Notably, if wood specific density was the only factor controlling species differences, stem-$CH_4$ emissions from *Alnus glutinosa* should have exceeded that of *Betula pubescens* at all times (although this was the case when the emissions through the year were pooled together). Instead, stem-$CH_4$ fluxes were greater for *Betula pubescens* than *Alnus glutinosa* both in summer and during the one-off sampling from additional trees in August, suggesting no

single factor exerts a dominant control on emission characteristics in these two tree species.

## 4.6. Conclusions

The results of this study indicate that tree-mediated $CH_4$ emissions are not simply a function of the concentration of $CH_4$ dissolved in pore-water and temperature but are far more complex. Tree-mediated $CH_4$ emissions contributed up to 27% to ecosystem $CH_4$ flux, with significant stem-$CH_4$ emissions observed even during the leafless season and emissions from young trees exceeding that of mature trees by orders of magnitude. These results therefore highlight that further work is essential to accurately measure and fully integrate this emission pathway into the ecosystem and global $CH_4$ budget. Furthermore, the response of tree-mediated $CH_4$ emissions in a changing environment (e.g., increased rainfall, thawing permafrost and increasing atmospheric $CO_2$) warrants further investigation because studies suggest that warming northern latitudes have resulted in enhanced tree growth and colonisation (Hartley *et al.,* 2012), positively affecting carbon mineralisation (both new and old recalcitrant carbon; Dorrepaal *et al.,* 2009), and ultimately $CH_4$ production. Therefore, further studies on the mechanistic understanding of all $CH_4$ emission pathways including tree-mediated $CH_4$ emission in forested wetland are imperative if we are to increase our knowledge of $CH_4$ dynamics in wetlands and its responses to climate change.

# CHAPTER FIVE

# Trees are Major Conduits for Methane Egress from Tropical Forested Wetlands

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## 5.1. Abstract


- Wetlands are the largest source of $CH_4$ to the atmosphere, with tropical wetlands comprising the most significant global wetland source component. The stems of some wetland adapted tree species are known to facilitate egress of $CH_4$ from anoxic soil, but current ground-based flux chamber methods for determining $CH_4$ inventories in forested wetlands neglect this emission pathway, and consequently, the contribution of tree-mediated emissions to total ecosystem $CH_4$ flux remains unknown.
- This study quantified *in situ* $CH_4$ emissions from tree stems, soil surfaces (ponded hollows and hummocks) and root-aerating pneumatophores in a tropical forested wetland in SE Asia.

- The study showed that tree stems emit substantially more $CH_4$ than soil surfaces, accounting for 62-87% of total ecosystem $CH_4$ flux. Tree stem flux strength was correlated with the stem diameter, wood specific density and the pore-water $CH_4$ concentrations.
- The study highlights the need to integrate this emission pathway in both field studies and models if wetland $CH_4$ fluxes are to be characterised accurately in global $CH_4$ budgets, and the discrepancies that exist between field-based flux inventories and top-down estimates of $CH_4$ emissions from tropical areas are to be reconciled.

## 5.2. Introduction

Natural wetlands are the single largest source of atmospheric $CH_4$ and are known to contribute significantly to interannual variations in the growth rate of this potent greenhouse gas (Hodson *et al.,* 2011). Gas transport through herbaceous plants adapted to wet soil is well documented (Brix *et al.,* 1992; Whiting & Chanton, 1996) and enables ingress of $O_2$ to the root zone but coincidental venting of soil-produced $CH_4$ to the atmosphere. Plant stems are a particularly efficient means for release of $CH_4$ from wetland soil because the pathway bypasses highly active populations of methanotrophic bacteria situated at the oxic-anoxic interface in the subsurface.

Trees also have the capacity to cope with soil anoxia through development of morphological adaptations such as hypertrophied lenticels, adventitious roots and enlarged aerenchyma. These structures promote gas exchange between the atmosphere and the rhizosphere (Megonigal & Day, 1992; Kozlowski, 1997), in particular, entry of $O_2$ to the root zone. Recent studies have demonstrated that temperate zone trees adapted to wet soil

also facilitate egress of soil-produced $CH_4$ (Rusch & Rennenberg, 1998; Vann & Megonigal, 2003; Terazawa *et al.,* 2007; Gauci *et al.,* 2010; Rice *et al.,* 2010) via gas transport through aerenchyma tissue and emission to the atmosphere through stem lenticels. Tropical mangroves similarly possess specialised aerial roots (pneumatophores) to transport atmospheric $O_2$ to submerged roots, which also release sedimentary $CH_4$ to the atmosphere (Purvaja *et al.,* 2004; Chauhan *et al.,* 2008). However, mangroves occupy sulphate-rich intertidal zones, accounting for only *c.* 0.7% of tropical forested area (Giri *et al.,* 2011), and consequently, $CH_4$ flux from mangroves globally is small (1.95 Tg $CH_4$ $a^{-1}$; Chauhan *et al.,* 2008) relative to other wetland sources.

Regardless, the capacity for wet soil-adapted trees to mediate $CH_4$ emissions has been demonstrated unequivocally by studies of mangroves and temperate forested wetlands (Rusch & Rennenberg, 1998; Vann & Megonigal, 2003; Purvaja *et al.,* 2004; Terazawa *et al.,* 2007; Chauhan *et al.,* 2008; Gauci *et al.,* 2010; Rice *et al.,* 2010). Notably, the same morphological adaptations to wet soil conditions are common in trees that inhabit vast areas of highly productive freshwater swamp and peatland at low latitudes (Kozlowski, 1997; Parolin *et al.,* 2006), yet measurements of $CH_4$ emission from tropical forested wetlands typically focus on fluxes from the ground surface collected using closed chambers (Jauhiainen *et al.,* 2005; Couwenberg *et al.,* 2010). Upscaling of field measurements that exclude tree-mediated $CH_4$ emissions may result in a significant underestimation of tropical $CH_4$ fluxes. Moreover, the absence of the tree-mediated $CH_4$ emission pathway in process-based models potentially limits their capacity to predict changes in trace gas exchange at the ecosystem level caused by internal or external perturbations.

Given that tropical wetlands account for the largest proportion of $CH_4$ flux from global wetlands and that *c.* 53% of these ecosystems are forested (Matthews & Fung, 1987; Prigent *et al.,* 2007), this study aimed to assess the extent to which trees may mediate $CH_4$ export from anoxic soil in tropical wetlands and evaluate their contribution to ecosystem emissions relative to other $CH_4$ emission pathways. *In situ* measurements of $CH_4$ flux through trees and from the ground surface conducted in a tropical forest wetland in Central Kalimantan (Indonesia, Borneo), SE Asia are presented here. Tropical forested wetlands of SE Asia are a significant reservoir of terrestrial organic carbon, storing *c.* 77% of tropical peatland carbon (Page *et al.,* 2011). High rates of precipitation lead to elevated water-table levels, resulting in slow decomposition rates that favour both peat accumulation and $CH_4$ production under anoxic conditions. Although significant quantities of $CH_4$ are produced in the peat, $CH_4$ typically is not released at high rates from the peat surface to the atmosphere because methanotrophic bacteria oxidize $CH_4$ at the oxic–anoxic interface in soil and within the rhizosphere (Couwenberg *et al.,* 2010). This study evaluated the extent to which trees in the wetland ecosystem function as conduits, enabling $CH_4$ to bypass soil methanotrophs, thereby facilitating its release to the atmosphere. To my knowledge, this is the first study to measure tree-mediated $CH_4$ emissions from tropical peat forests and also the first to estimate the contribution of trees to total ecosystem $CH_4$ flux.

## 5.3. Materials and Methods

Methane fluxes from tree stems, the soil surface (ponded hollows and hummocks) and root-aerating pneumatophores were measured during a 2-week period in March 2011 in two 20 × 20 m (400 $m^2$) plots during the wet season in a tropical forested peatland situated

in the upper Sebangau River catchment in Borneo, Indonesia. Full description of the site can be found in Chapter 2 (section 2.2.2).

Static chambers used to measure $CH_4$ fluxes from soil surface (hollow and hummocks) are described in Chapter 2 (section 2.3.3). Approximately 30 fluxes were measured from each hollow and hummock per plot (i.e., 120 measurements in total). Static chambers used to measure $CH_4$ fluxes from tree stems are described in Chapter 2 (Fig. 2.8; section 2.3.1) Stem-$CH_4$ emissions were measured twice from a minimum of four trees per species (stem diameter, 7.5-19.5 cm) for the eight dominant tree species chosen randomly within each plot. The eight dominant species within the two plots were: *Mesua* sp. 1, *Xylopia fusca*, *Shorea balangeran*, *Diospyros bantamensis*, *Tristaniopsis* sp. 2, *Litsea elliptica*, *Elaeocarpus mastersii* and *Cratoxylum arborescens*. These tree species accounted for *c.* 72% of all trees within the two plots. Methane emissions from tree stems were measured at three intervals between 20 and 130 cm height above the peat surface. All samples were stored and transported in 12 ml pre-evacuated Exetainer vials (Labco Ltd, High Wycombe, UK) and analysed as described in Chapter 2 (section 2.5).

An increment borer was used to extract wood samples at stem heights of 35, 75, 115 and 130 cm from the eight tree species and the specific density of the wood was calculated as described in Chapter 2 (section 2.7). Pore-water samples were extracted at three soil depths (50, 100 and 150 cm below the soil surface) within the two study plots at two locations in the tropical forested wetland. Further details on the pore-water samplers can be found in Chapter 2 (section 2.4.2).

*5.3.1. Statistical analysis*

All statistical analyses were conducted with SPSS software v.19 (SPSS, Chicago, IL, USA) using a significance level of $P < 0.05$. Datasets were tested for normal distribution using Shapiro-Wilko test. A general linear model (ANOVA repeated measures) along with Tukey's HSD test ($P \leq 0.05$) was used for comparison of means. Relationships between stem-$CH_4$ fluxes, stem diameter, stem sampling height and wood specific density were evaluated using regression models. The relative contributions of independent variables (stem diameter, wood specific density and pore-water $CH_4$ concentrations) to stem-$CH_4$ fluxes at different stem heights were determined using multiple regression analysis. All independent variables were first tested for multicollinearity and homoscedasticity.

## 5.4. Results and Discussion

Seven of the eight tree species released significant quantities of $CH_4$ from their stems (Fig. 5.1), with fluxes ranging from 17.0 ± 1.4 to 185 ± 7 μg $CH_4$ $m^{-2}$ $h^{-1}$on a stem surface area basis. Measurable stem emissions were not observed from *Cratoxylum arborescens*, the least prevalent tree species of the eight studied within the plots. The rate of $CH_4$ flux significantly decreased ($P < 0.001$) with stem height above the forest floor in all species (Fig. 5.1). Emissions from the soil surface averaged 32.9 ± 7.8 μg $CH_4$ $m^{-2}$ $h^{-1}$ for hollows and 0.7 ± 0.5 μg $CH_4$ $m^{-2}$ $h^{-1}$ for hummocks. In both study plots, stem-$CH_4$ fluxes measured from 20 to 50 cm stem heights on each tree were larger than soil surface $CH_4$ fluxes. The three dominant tree species in the plots (*Shorea balangeran*, *Mesua* sp. 1 and *Xylopia fusca*) had the highest rates and *Elaeocarpus mastersii* had the lowest average rate of $CH_4$ egress. Stem-$CH_4$ flux rates from *Diospyros bantamensis*, *Tristaniopsis* sp. 2 and *Litsea elliptica* were similar in magnitude and not statistically different.

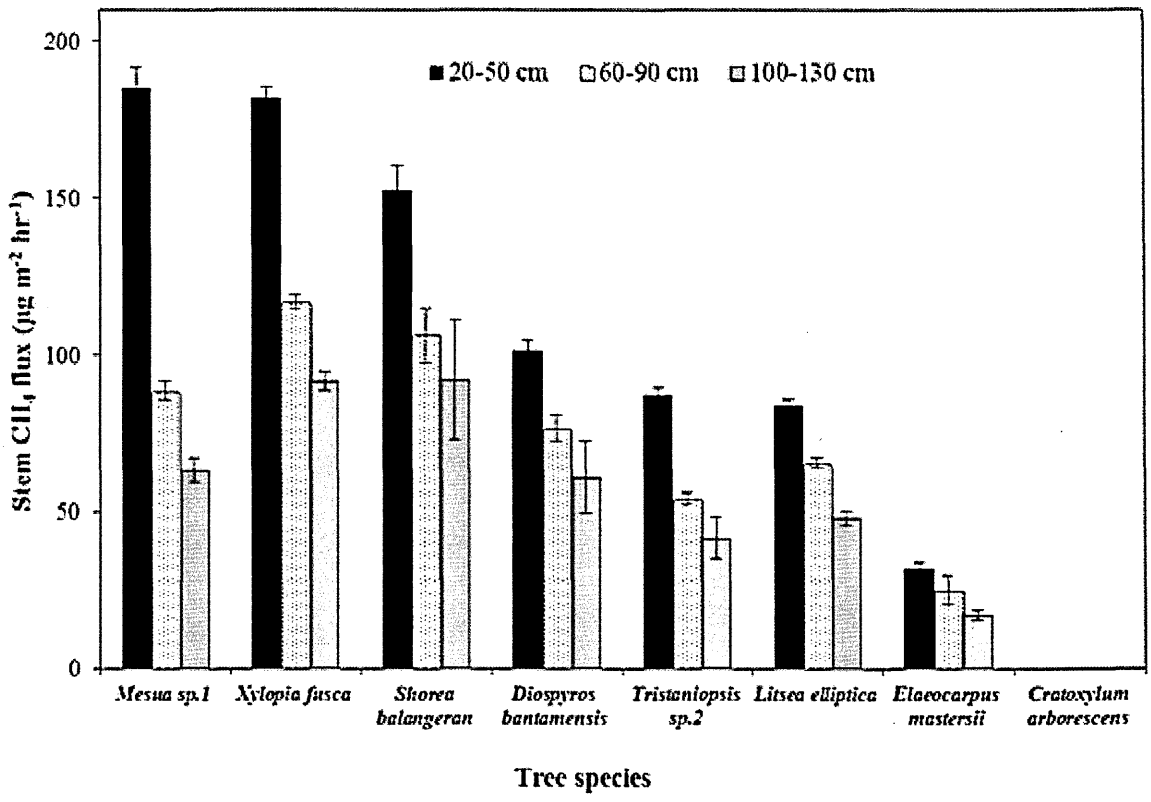


Figure 5.1: Mean tree stem-$CH_{4p}$ fluxes (± SE, n ≥ 4 trees per species) from tree species along three stem height positions (20 to 50 cm, 60 to 90 cm and 100 to 130 cm above soil surface).

Stem cores extracted across a range of stem heights in a subset of trees within each plot displayed no evidence of heartwood rot, which can result in $CH_4$ production within trees (Zeikus & Ward, 1974; Covey *et al.,* 2012). This observation, coupled with the finding that $CH_4$ emissions decreased with height above the forest floor for all trees studied (Fig. 5.1) and the presence of significant concentrations of $CH_4$ dissolved in soil water in the plots (113–539 μmol $l^{-1}$ at 50–150 cm soil depth), indicates that the anoxic peat soil was the main source of stem-emitted $CH_4$, minimising the likelihood of any substantial cryptic sources (e.g., tree holes; Martinson *et al.,* 2010). The presence of an extensive root network

reaching the $CH_4$ production zone and a well-connected root-stem path for the transport of $CH_4$ are prerequisites for this hypothesis.

The stem diameter and wood densities at breast height (1.3 m) of the eight tree species studied are listed in Table 5.1. Stem-$CH_4$ fluxes varied significantly between seven tree species studied ($P < 0.0001$) and at three stem height positions ($P < 0.001$). Stem-$CH_4$ fluxes from all seven tree species exhibiting a significant relationship with stem diameter ($R^2 = 0.38$; $P < 0.001$; Fig. 5.2a) and wood specific density ($R^2 = 0.47$; $P < 0.0001$; Fig. 5.2b). Multiple regression analysis indicates that stem diameter, wood specific density and pore-water $CH_4$ concentrations explain up to 80% ($R^2 = 0.808$; $P < 0.0001$) of stem-$CH_4$ flux variations (Table 5.2). These relationships were observed for fluxes measured at all stem heights (20–50, 60–90 and 100–130 cm above the soil surface). Stem diameter and wood specific density were inversely related to stem-$CH_4$ flux, whereas pore-water $CH_4$ concentrations were positively related to stem-$CH_4$ emission rates (Table 5.2). The latter relationship is consistent with findings from previous studies (Rusch & Rennenberg, 1998; Terazawa *et al.,* 2007), but the observation of an inverse relationship between stem-$CH_4$ flux and diameter and wood specific density has not been reported to date. Notably, wood specific density is a well-known indicator of the functional traits and properties of wood, including porosity and anatomical composition, and varies within individual trees and between trees, commonly being influenced by ecophysiological factors such as flooding (Parolin & Worbes, 2000; Wittmann *et al.,* 2006a, b). Therefore, the lack of any measurable $CH_4$ emissions from *Cratoxylum arborescens* was probably a result of stem properties in the tree with larger stem diameter and higher wood specific density than other trees in this study, but may also have been a result of root distribution (i.e., roots failing to reach the $CH_4$ production zone) and/or differences in transport pathways and $CH_4$ egress

points (e.g., $CH_4$ transport through the transpiration stream and release via leaf surfaces that were not measured here).

Table 5.1: Tree diameter (DBH ≥ 7cm) and wood specific density measured at 1.3 m stem height above soil surface for the eight tree species studied.

| Tree species studied | DBH range (cm) | Wood specific density range (g $cm^{-3}$) |
|---|---|---|
| *Shorea balangeran* | 7.5-12.4 | 0.428-0.517 |
| *Elaeocarpus mastersii* | 12-15.6 | 0.601-0.828 |
| *Diospyros bantamensis* | 9.2-15.5 | 0.489-0.581 |
| *Litsea elliptica* | 9-13.8 | 0.601-0.801 |
| *Tristaniopsis* sp. 2 | 10.7-13 | 0.506-0.746 |
| *Mesua* sp. 1 | 10.8-14.2 | 0.545-0.607 |
| *Xylopia fusca* | 8.9-11.4 | 0.435-0.551 |
| *Cratoxylum arborescens* | 12.6-19.8 | 0.635-0.801 |

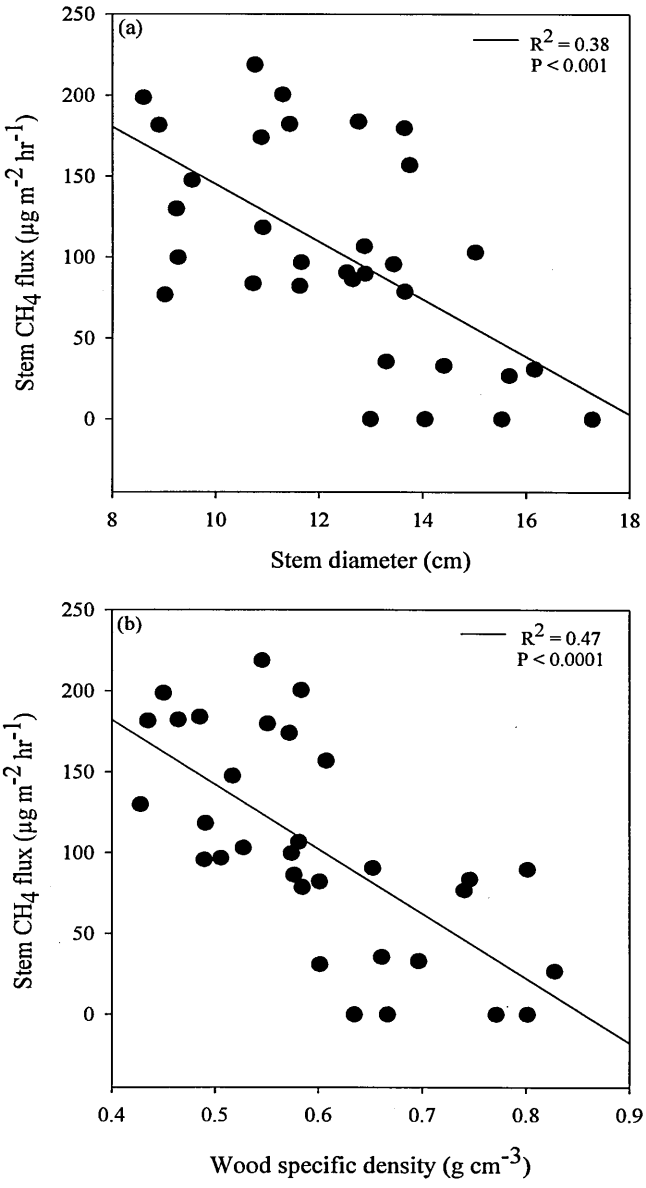


Figure 5.2: Relationship between stem-$CH_4$ flux and a) stem diameter and b) wood specific density measured at 20-50 cm above the peat surface. The regression equations are: a) Y = 322.7 – 17.75 × (stem diameter), and b) Y = 342.01 – 399.52 × (wood specific density).

Table 5.2: Results of multiple regression analysis of stem-$CH_4$ fluxes measured at three stem heights (20-50 cm, 60-90 cm and 100-130 cm above the soil surface), stem diameter and wood specific density measured at corresponding stem heights, and concentrations of $CH_4$ dissolved in pore-water at 50 cm below the soil surface measured within 2.5 m radius of the trees under investigation.

| | 20-50 cm | | 60-90 cm | | 100-130 cm | |
|---|---|---|---|---|---|---|
| | Coefficients | Standard Error | Coefficients | Standard Error | Coefficients | Standard Error |
| Adjusted $R^2$ | 0.808 ($P < 0.0001$) | | 0.764 ($P < 0.0001$) | | 0.693 ($P < 0.0001$) | |
| Intercept | 345 ($P < 0.0001$) | 37.9 | 239 ($P < 0.0001$) | 26 | 154 ($P < 0.0001$) | 20.8 |
| Stem diameter (cm) | -11.2 ($P = 0.002$) | 3.2 | -8.27 ($P = 0.002$) | 2.26 | -4.57 ($P = 0.02$) | 1.81 |
| Wood specific density (g $cm^{-3}$) | -323 ($P < 0.001$) | 69.3 | -190 ($P = 0.0008$) | 48.2 | -151 ($P = 0.001$) | 39.2 |
| Pore-water concentration (μmol $l^{-1}$) | 0.646 ($P < 0.001$) | 0.165 | 0.253 ($P = 0.049$) | 0.121 | 0.263 ($P = 0.02$) | 0.1 |

Power function relationships between the rate of stem-$CH_4$ emission and stem sampling height were determined for five of the seven tree species (Table 5.3), suggesting that the entire tree may release $CH_4$, albeit at much lower rates from higher portions. Methane emission rates along the length of trees were estimated using regression models based upon the power function relationships; however, $CH_4$ fluxes from only the 0.1 to 3 m bottom section of tree stems were used to determine a conservative estimate of tree-mediated $CH_4$ emissions in the ecosystem flux calculations, pending direct measurement and confirmation of $CH_4$ emissions from higher portions of trees.

Table 5.3: Relationship between stem-$CH_4$ fluxes and stem sampling position above the forest floor (20-50 cm, 60-90 cm and 100-130 cm above the soil surface) for the seven of the eight tree species studied that released $CH_4$. y = average stem-$CH_4$ flux ($\mu g\ m^{-2}\ hr^{-1}$) for each 30 cm section of the tree that was measured; x = average stem height (cm) of that 30 cm section.

| Tree species studied | Relationship | $R^2$ |
|---|---|---|
| *Shorea balangeran* | $y = 703(x^{-0.432})$ | 0.991 |
| *Elaeocarpus mastersii* | $y = -0.184x + 38.4$ | 0.998 |
| *Diospyros bantamensis* | $y = 455(x^{-0.42})$ | 0.992 |
| *Tristaniopsis* sp. 2 | $y = 785(x^{-0.619})$ | 0.999 |
| *Mesua* sp. 1 | $y = 4630(x^{-0.909})$ | 0.996 |
| *Litsea elliptica* | $y = -0.445x + 99.1$ | 0.999 |
| *Xylopia fusca* | $y = 1410(x^{-0.577})$ | 0.999 |

Soil surface $CH_4$ fluxes per plot were estimated after deducting tree basal area and using a 50:50 proportion of hollow vs. hummock coverage. The conservative estimate of total tree-mediated $CH_4$ flux per plot (i.e., considering only the lowermost 3 m of tree emissions) is

6.7 ± 0.7 g $ha^{-1}$ $d^{-1}$, which is approximately twice the flux from hollows (3.9 ± 1.0 g $ha^{-1}$ $d^{-1}$; Fig. 5.3) and *c.* 62% of total ecosystem flux and the largest contributor of $CH_4$ to the atmosphere from this ecosystem. Inclusion of tree emissions to an average height of 15 m based upon the power function relationships yields total tree-mediated $CH_4$ emissions of 28.5 ± 3.4 g $ha^{-1}$ $d^{-1}$ or *c.* 87% of total ecosystem flux. These findings suggest that exclusion of $CH_4$ emissions from tree stems in field studies that use only ground chambers to measure $CH_4$ flux in forested tropical wetlands may result in significant underestimation of total $CH_4$ emissions from the ecosystem.

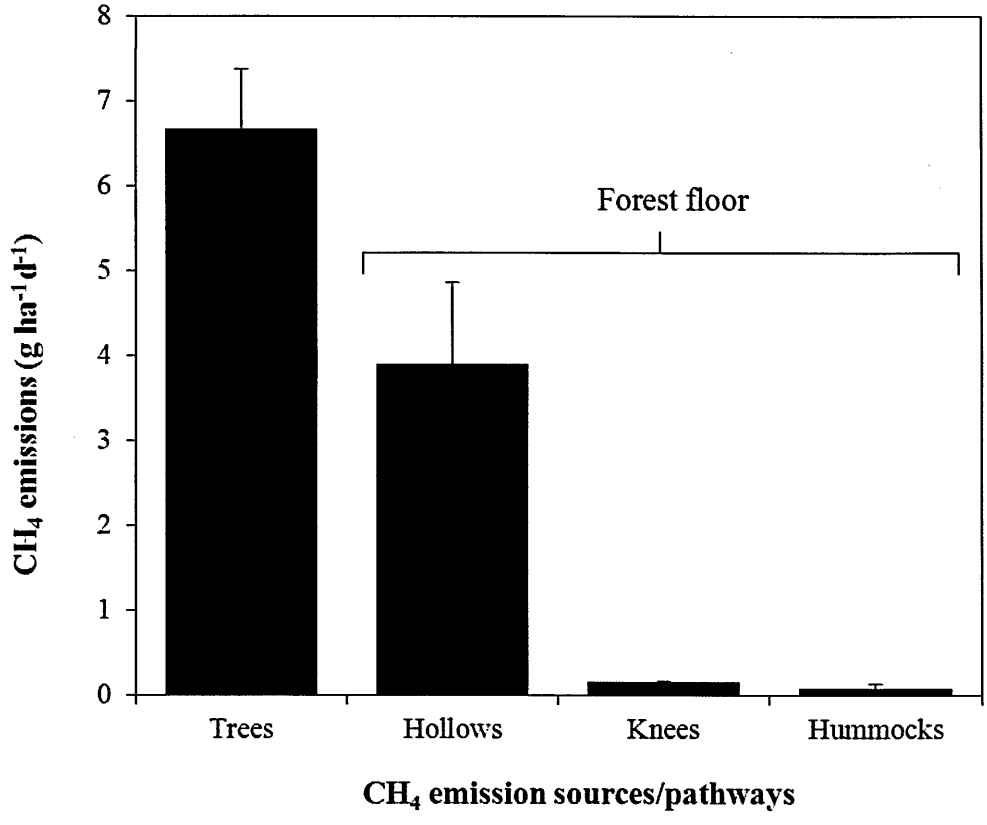


Figure 5.3: Estimated total $CH_4$ emissions (± SE) from hollows, hummocks, root-aerating pneumatophores (knees) and tree stems. Regression models of $CH_4$ emission versus tree height were applied to a maximum of 3 m of the bottom-most stem height (average tree height ~15 m).

The study findings are likely also to be of relevance to other tropical forested wetlands beyond SE Asian tropical peat forests, which only account for *c.* 10% of forested tropical wetlands globally. Tropical peat forests in SE Asia are known to emit less $CH_4$ than nutrient-rich tropical wetlands (Wassmann *et al.,* 1992), because, in the latter soil, pH is higher (Bartlett *et al.,* 1988; Koschorreck, 2000), $CH_4$ production is greater and methanotrophy is generally less effective due to increased anoxic and stratified-water submerged sediments (Bartlett *et al.,* 1988; Devol & Rickey, 1990; Koschorreck, 2000) resulting from higher water-table levels. Within seasonally inundated wetlands, soils are submerged for prolonged periods and water column productivity contributes labile biomass to bottom sediment (Devol & Rickey, 1990 and references within) resulting in greater $CH_4$ production. The relative proportions of $CH_4$ flux via tree stems, the soil surface, herbaceous plants, and ebullition (i.e., release of $CH_4$-rich gas bubbles) will almost certainly differ in other types of forested tropical wetland both spatially and seasonally, depending upon moisture regime. However, there are key similarities between all forested tropical wetlands that are likely to ensure a significant role for wetland-adapted trees in mediating $CH_4$ flux.

First, the development of morphological adaptations to aerate root systems is a common feature in trees that inhabit seasonally or permanently wet soil (Kozlowski, 1997; Parolin *et al.,* 2006). To date, the majority of tree species investigated that possess adaptive structures to facilitate $O_2$ ingress also are capable of mediating $CH_4$ egress (Rusch & Rennenberg, 1998; Vann & Megonigal, 2003; Purvaja *et al.,* 2004; Terazawa *et al.,* 2007; Gauci *et al.,* 2010; Rice *et al.,* 2010). Notably, six of the eight tree species investigated in this study in Borneo belong to families that are widely distributed amongst Amazonian wetlands (Elaeocarpaceae (*Elaeocarpus mastersii*), Ebenaceae (*Diospyros bantamensis*), Myrtaceae (*Tristaniopsis sp.* 2), Clusiaceae or Guttiferae (*Mesua sp.* 1), Lauraceae (*Litsea*

*elliptica*), Annonaceae (*Xylopia fusca*); (Parolin *et al.,* 2006; Wittmann *et al.,* 2006a, b; Saatchi *et al.,* 2007; Macía, 2011). Also, the wood specific densities of the related Amazonian wetland tree species correspond with the range reported in this study (0.22–0.87 g $cm^{-3}$, Parolin & Worbes, 2000; Wittmann *et al.,* 2006a, b). Moreover, it is well established that trees inhabiting Amazonian varzeas generally exhibit morphological adaptations that facilitate gas transport during periods of inundation (Parolin *et al.,* 2006; Graffmann *et al.,* 2008). Hence, there is considerable evidence to suggest that most wetland-adapted trees possess structures that enable $CH_4$ egress from soil.

Second, wetland-adapted trees do not appear to be limited in their capacity to transport $CH_4$ (Rusch & Rennenberg, 1998; Chapter 3), but rather the amount of $CH_4$ present in the subsurface is a more critical factor determining rates of $CH_4$ flux from tree stems (Rusch & Rennenberg, 1998; Terazawa *et al.,* 2007; Rice *et al.,* 2010). Mesocosm experiments on common alder saplings by Rusch & Rennenberg (1998) demonstrate a strong positive linear relationship between $CH_4$ concentrations in the root zone and stem-$CH_4$ fluxes. Notably, rates of $CH_4$ egress from tree stems in mesocosms greatly exceed *in situ* flux rates measured in this study, because rhizosphere concentrations of $CH_4$ are artificially elevated in the mesocosm studies. In SE Asian tropical peat forest, pore-water from 0 to 50 cm depth contained a maximum concentration of 123 μmol $l^{-1}$. The amount of $CH_4$ in deeper peat in the Borneo peatland was greater (113–1539 μmol $l^{-1}$ from 50 to 150 cm depth); however, *c.* 83% of root biomass occurs within 0–30 cm depth in the tropical peat forest and root abundance decreases exponentially with depth (Sulistiyanto *et al.,* 2004; Jauhiainen *et al.,* 2005; Verwer & van der Meer, 2010). By contrast, more nutrient-rich tropical wetlands typically contain higher concentrations of $CH_4$ in shallow pore-water. For example, shallow soil (0-30 cm depth) in Amazonian wetlands has been reported to contain dissolved $CH_4$ concentrations of 175-1380 μmol $l^{-1}$ (Bartlett *et al.,* 1988; Koschorreck,

2000). High concentrations of $CH_4$ in shallow soil are particularly common where standing water is present, because it impedes entry of $O_2$ to support methanotrophy (Bartlett *et al.*, 1988; Koschorreck, 2000). Ebullition may become an important pathway under such conditions (Bartlett *et al.*, 1988; Wassmann *et al.*, 1992; Koschorreck, 2000); however, high concentrations of $CH_4$ at shallow depths, coupled with low $O_2$ concentrations and the need for trees to aerate their root zone, present all the elements required for tree-mediated $CH_4$ flux.

While the results of this study demonstrate that there is significant potential for tree-mediated $CH_4$ emission in other types of tropical forested wetlands, the actual contribution of $CH_4$ export via trees to total ecosystem flux remains unknown. The majority of ground-based $CH_4$ emission studies in tropical wetlands have been conducted using soil chambers and, as a result, tree-mediated $CH_4$ fluxes are absent in scaled surface estimates of $CH_4$ emissions. Notably, characterisation of tree-mediated $CH_4$ fluxes in other types of tropical forested wetland may help to reconcile discrepancies that currently exist between scaled ground-based $CH_4$ fluxes and an unexplained excess of tropical atmospheric $CH_4$ observed in atmospheric and space-borne measurements (Chen & Prinn, 2005; Miller *et al.*, 2007; Frankenberg *et al.*, 2008). The findings of this study may be particularly important given that other tropical $CH_4$ sources suggested recently to account for the inconsistency between bottom-up and top-down inventories have been shown to be negligible globally (e.g., UV-driven aerobic fluxes from plants (Bloom *et al.*, 2010) and tank bromeliads in tree canopies (Martinson *et al.*, 2010)).

Process-based global emission models simulate $CH_4$ production as a function of net primary productivity (NPP) and respiration (Walter & Heimann, 2000) and thus implicitly include emissions derived from productivity and decomposition processes in forests

(Spahni *et al.,* 2011). Such models typically generate $CH_4$ emission estimates that are larger than scaled field measurements and which are more similar to estimates derived from inverse methods (Spahni *et al.,* 2011). However, process-based models at present do not discriminate between herbaceous and tree-mediated transport of $CH_4$ (Walter *et al.,* 2001) and some do not define pathways by which soil-produced $CH_4$ is exported to the atmosphere (Spahni *et al.,* 2011). Moreover, current models are parameterised based upon $CH_4$ flux measurements from low herbaceous wetland canopies (Walter & Heimann, 2000) and consequently may not respond correctly when subjected to different environmental stimuli. For example, tropical forests possess dense multi-layered canopies that are sensitive to variation in diffusive light; small increases in incident light intensities on normally shaded leaves stimulate NPP (Mercado *et al.,* 2010), whereas no such interaction exists in northern wetlands dominated by short shrubs (Letts *et al.,* 2005). If tree-mediated $CH_4$ fluxes are a dominant contributor to ecosystem $CH_4$ emissions from tropical forested wetlands, as suggested by this study, then there is a need for explicit inclusion of trees and relevant physiological responses in process-based emission models otherwise the capacity for such models to predict the effects of environmental change on trace gas fluxes may be limited. Accurate modelling of interannual variability in $CH_4$ emissions and the long-term effects of climate change on $CH_4$ fluxes from the tropics may rely upon parameterisation of subtle responses of wetland-adapted trees to moisture and temperature.

Finally, current protocols for $CH_4$ measurement in forested wetlands may require revision if we are to reduce uncertainties in global $CH_4$ source estimates and provide accurate accounting of greenhouse gas exchange under different land-use scenarios (with potential economic consequences under the United Nation's Reducing Emissions from Deforestation and Forest Degradation (REDD) programme). The role of trees in the $CH_4$ cycle should not, however, excuse deforestation, because tree-mediated $CH_4$ flux measured

in this study, when expressed in $CO_2$ equivalents represents < 2% of total carbon emissions from deforested tropical peat forests (Hirano *et al.,* 2007). Foremost, this study underscores the need for further study of tree-mediated $CH_4$ emissions to determine whether wetland-adapted trees normally dominate ecosystem $CH_4$ fluxes in all types of forested tropical wetland.

# CHAPTER SIX

# Discussion and Synthesis

## 6.1. Introduction

The research presented in this thesis primarily investigated the role of tree-mediated $CH_4$ emission pathway relative to other well-known $CH_4$ emission pathways in a temperate and tropical forested wetland and assessed their contributions to net ecosystem $CH_4$ flux. This chapter discusses the implications of this research by re-examining the objectives presented in Chapter 1 and synthesising findings from all chapters. Recommendations for further studies also are presented.

One of the important outcomes of this study is that it demonstrates that mature trees in both temperate and tropical regions have the ability to transport $CH_4$ produced in soil to the atmosphere and contribute significantly to ecosystem $CH_4$ flux. This study provides new insights into the controls and variability of tree-mediated $CH_4$ emissions and lays the foundation of work in an area where still little is known.

## 6.2. Obj.1. To assess the presence or absence of tree-mediated $CH_4$ emissions from wetland-adapted trees (both tropical and temperate)

Objective 1 was evaluated in Chapter 3 (mesocosms experiment), Chapter 4 (temperate forested wetland) and Chapter 5 (tropical forested wetland). Data reported in those chapters demonstrated significant $CH_4$ release through stems of wetland-adapted trees. Although the magnitude (0-216 $\mu g\ m^{-2}\ hr^{-1}$) and overall ecosystem contributions (6-87%) varied between the two ecosystems, the results conclusively demonstrate that trees adapted to wet soil can mediate release of significant quantities of soil-produced $CH_4$ to the atmosphere.

This study, together with previous studies, confirms tree-mediated $CH_4$ release from nine temperate tree species (*Alnus glutinosa, Betula pubescens, Fraxinus latifolia, Populus trichocarpa, Salix fluviatilis, Taxodium distichum, Fraxinus mandshurica var. japonica, Populus deltoides* × *Populus nigra* and *Fagus sylvatica*) and for the first time, from seven tropical tree species (*Mesua* sp. 1, *Xylopia fusca*, *Shorea balangeran*, *Diospyros bantamensis*, *Tristaniopsis* sp. 2, *Litsea elliptica* and *Elaeocarpus mastersii*). Nine of the ten tree species investigated in this study (temperate and tropical forested wetland combined) released significant quantities of $CH_4$ from stem surfaces. Two tree species in the temperate region, *Alnus glutinosa* and *Betula pubescens*, released $CH_4$ through their stem surfaces year round, including winter months. The exception was *Cratoxylum arborescens*, a less-dominant tree species of SE Asian forested wetland. The reason for the absence of $CH_4$ emissions from *Cratoxylum arborescens* is unclear, but it could be due to one of the reasons outlined in Chapter 5 (section 5.4; page no: 124). However, the broader significance of a lack of $CH_4$ emissions from *Cratoxylum arborescens* is that it demonstrates that not all trees adapted to wetland environments release $CH_4$.

Methane emissions from leaf surfaces were not measured from mature trees *in situ*, however, studies conducted in tropical and temperate forested wetland and in mesocosms provide strong evidence to suggest that $CH_4$ flux through leaf surfaces, if present, would be small or insignificant. The mesocosm experiment conclusively demonstrates that stem surfaces are the principal point of $CH_4$ egress from young *Alnus glutinosa.* Methane emissions through leaf surfaces were not detected (Chapter 3) and stem-$CH_4$ emissions when scaled to the entire tree yielded values similar to tree-mediated $CH_4$ emissions (estimated by subtracting whole-mesocosm $CH_4$ emissions and soil $CH_4$ emissions; Chapter 3; Table 3.2), thus highlighting the dominance of stem-$CH_4$ emissions. While such direct evidence was absent *in situ*, the following observations favour the conclusion that only small quantities of $CH_4$ may reach tree heights where leaves are dominant resulting in insignificant $CH_4$ emission from leaf surfaces: i) the decrease in stem-$CH_4$ emissions with increasing stem height, which suggests diffusion of $CH_4$ via a concentration gradient, and that the gradient and consequently diffusion, decrease with height (Fig. 5.1; Tables 4.1, 4.2 and 5.3); ii) the increase in wood specific density with increasing stem height, which suggests that the volume of tissues/pore spaces aiding $CH_4$ transport decreases with increasing stem height (Table 4.5); and iii) the small contribution from transpiration-driven $CH_4$ transport mechanism suggesting a lack of long distance $CH_4$ transport (discussed in section 6.4).

Rates of stem-$CH_4$ emission varied between tree species within and between ecosystems (tropical and temperate forested wetlands). However, the maximum rates of stem-$CH_4$ emission observed from mature trees in both ecosystems (during the 2-week campaign in tropical forested wetland and summer emissions in temperate forested wetland) were of similar magnitude (210 $\mu g\ m^{-2}\ hr^{-1}$ vs. 290 $\mu g\ m^{-2}\ hr^{-1}$) despite the pore-water $CH_4$ concentrations and $CH_4$ dynamics in the soil varying greatly between the two ecosystems.

Maximum rates of stem-$CH_4$ emissions reported in the literature (170 μg $m^{-2}$ $hr^{-1}$; Terazawa *et al.,* 2007) do not exceed the rates reported in this study. Given that only three studies (including this one) have investigated stem-$CH_4$ flux from mature trees, future studies in nutrient-rich tropical wetlands (e.g., Amazonian wetland) would offer further insights. Observations so far suggest a possible maximum capacity of $CH_4$ transport in mature trees.

Mesocosm experiments conducted using young *Alnus glutinosa* exposed to enriched pore-water $CH_4$ concentrations (603 - 908 μmol $l^{-1}$; Chapter 3; Table 3.5), provide evidence in favour of continued and increased stem-$CH_4$ emission under increased soil $CH_4$ concentration without reaching a threshold. Similar continued and increased stem-$CH_4$ emissions were also found by Rush & Rennenberg (1998) and Rice *et al.* (2010). Both these studies evaluated tree-mediated $CH_4$ emissions from young trees under elevated soil $CH_4$ concentrations. Therefore, it appears that, at least in young trees, there may not be a tree physiological limitation on rates of $CH_4$ transport. However, several orders of magnitude difference in stem-$CH_4$ emissions observed between young versus mature *Alnus glutinosa* and *Betula pubescens* in temperate forested wetland (Fig. 4.2), which experienced similar pore-water $CH_4$ concentrations, highlight the possibility of physiological development differences controlling stem-$CH_4$ emissions, which requires further investigation.

### 6.3. Obj.2. To assess the spatial and temporal variability of $CH_4$ emissions along the height of the tree and between different trees species.

Objective 2 was evaluated in Chapters 3, 4 and 5 and results suggest that stem-$CH_4$ emissions vary between the wetland ecosystems studied (Chapters 4 and 5), along the

length of tree (Chapters 3, 4 and 5) and between tree species (Chapters 4 and 5). Stem-$CH_4$ emissions also varied over time in temperate forested wetland with species-specific differences (Chapter 4). The variations in stem-$CH_4$ emissions between tree-species have been discussed in Chapters 4 and 5 (temperate forested wetland, section 4.5; tropical forested wetland, section 5.4). The following sections discuss some common observations between the two ecosystems and implications of the temporal variations observed therein.

In both the ecosystems, significant stem-$CH_4$ emissions were observed along the length of the tree (130-170 cm above the soil surface) from nine of the ten mature tree species studied (Fig. 5.1), with stem-$CH_4$ emissions decreasing with increasing stem sampling height (Tables 4.1, 4.2 and 5.3). This stem-$CH_4$ emission pattern along the length of the tree, also observed by Rusch & Rennenberg (1998) and Terazawa *et al.* (2007), is opposite to that observed by Covey *et al.* (2012), where heartwood and wetwood rot were documented to contribute to most of the tree trunk $CH_4$ concentrations. Covey *et al.* (2012) reported lower tree trunk $CH_4$ concentrations at 5 cm stem height compared to 130 cm. However, the stem-$CH_4$ fluxes observed in this study were not produced within the tree trunk; the trees merely functioned as conduits for the release of soil-produced $CH_4$ as no visible rot was observed from any of the tree cores extracted from all ten mature tree species. The likely occurrence of heartwood and wetwood rot in trees > 25 cm stem in diameter reported in literature (Browne 1956; Berry & Beaton, 1972) offer additional evidence of lack of $CH_4$ production within the trees investigated here, as all trees had a stem diameter < 20 cm.

The height of the tree emitting $CH_4$, the relationship between stem-$CH_4$ emission and stem height and factors driving both the capacity and pattern of stem-$CH_4$ emissions along the length of the tree, are all important to assess the contributions of tree-mediated $CH_4$

emissions to ecosystem $CH_4$ flux. This study provides evidence of species-specific differences in the relationship between stem-$CH_4$ emission and stem height. Both power function and linear relationships between stem-$CH_4$ emissions and stem sampling height were observed. The latter relationship was observed for *Elaeocarpus mastersii* and *Litsea elliptica* in the tropical forested wetland (Chapter 5; Table 5.3) and for *Betula pubescens* in temperate forested wetland only in winter (Chapter 4; Table 4.1). All other trees displayed a power function relationship.

Results from Chapters 3, 4 and 5 suggest that variations in stem-$CH_4$ emission along the length of the tree may be largely ascribed to differences in $CH_4$ transport mechanisms (discussed in section 6.4), together with physiological and morphological parameters varying both within and between tree species (e.g., wood specific density (Chapters 4 and 5) and stem lenticel density (Chapter 3)). Notably, the temporal changes in stem-$CH_4$ emission patterns along the length of *Betula pubescens* (switching from power relations in summer, spring and autumn to linear relations in winter) may be primarily due to change in $CH_4$ transport mechanisms, i.e., a switch from a combination of diffusion and transpiration-driven/convective $CH_4$ transport to diffusion-driven transport mechanism alone, in winter. While, autumnal leaf loss should limit transpiration-driven $CH_4$ transport in both tree species, diurnal variation studies conducted *in situ* suggest that transpiration-driven/convective $CH_4$ transport was more important in *Betula pubescens* than *Alnus glutinosa*. Therefore autumnal leaf loss regulating transpiration-driven/convective $CH_4$ transport had a negligible impact on emission patterns from *Alnus glutinosa*, but had a significant effect on both the capacity and patterns of stem-$CH_4$ emissions along the length of *Betula pubescens*.

Stem-$CH_4$ emissions varied temporally, over both short (diurnal) and long (seasonal) time scales, due to temporal changes in several factors, including temperature (discussed in section 6.5), physiological and morphological parameters (discussed in section 6.4) and transport mechanisms (discussed in section. 6.4). Both diurnal and seasonal variations have important implications for the timing of flux measurements as short daytime and season specific measurements will result in under- or over-estimates of $CH_4$ emissions. For instance, daytime $CH_4$ emissions from *Betula pubescens* were 36.4% greater than at night, but this difference was only 13.8% in *Alnus glutinosa*. These results highlight up to 36.4% overestimation if diurnal variations are not considered. Similarly, rates of stem-$CH_4$ emission from *Betula pubescens* were 75% lower in winter than summer, but this difference was only 28% in *Alnus glutinosa*. Such differences in seasonal and diurnal patterns should be carefully measured and accounted for in all ecosystems.

Winter emissions from all $CH_4$ emission pathways accounted for only 9.2% of annual emissions. Interestingly, the reduced $CH_4$ contribution in winter was not because of lower stem-$CH_4$ emissions but was due to reduction in other non-tree $CH_4$ emission pathways (Chapter 4; Table 4.3). The percentage variation in the rates of stem-$CH_4$ emission between summer and winter was small compared to non-tree $CH_4$ emission pathways where winter fluxes from non-tree $CH_4$ emission pathways were several orders of magnitude lower than summer fluxes. Additionally, species-specific differences observed in seasonal and diurnal variations in stem-$CH_4$ emissions suggest that temporal variation patterns for stem-$CH_4$ emissions cannot be generalised at an ecosystem level, unless temporal variations from the majority of the tree species within an ecosystem are measured.

### 6.4. Obj.3. To investigate the mechanisms responsible for transport and release of $CH_4$ by wetland trees.

Although stem-$CH_4$ fluxes do not reveal the exact mechanisms of $CH_4$ transport, they are an indirect path to understanding $CH_4$ transport mechanisms through trees and therefore helped evaluate objective 3. Stem-$CH_4$ flux measured *in situ* (Chapters 4 and 5) and in mesocosms (Chapter 3) collectively suggests that soil-produced $CH_4$ is released to the atmosphere via tree stem lenticels predominantly by a diffusion-driven transport mechanism. Transpiration-driven $CH_4$ transport was found to be less significant. These aspects are discussed in detail below.

Decreasing stem-$CH_4$ emissions with increasing stem height and a strong positive and linear relationship between stem-$CH_4$ emissions and pore-water $CH_4$ concentrations observed in nine of the ten tree species studied offer evidence of diffusion-driven $CH_4$ transport following a concentration gradient between the root zone and atmosphere. These observations compare well with reports of Rusch & Rennenberg (1998) and Terazawa *et al.,* (2007), which show similar relationship between stem-$CH_4$ emissions and pore-water $CH_4$ concentrations. The mesocosm experiment conducted using *Alnus glutinosa* saplings offers additional evidence in favour of a diffusion-driven transport mechanism and are discussed in Chapter 3 (section 3.5.3; page no: 80-81).

Examining the fluxes measured at night in comparison to those during the day helps evaluate the role of different $CH_4$ transport mechanisms. Less than 13.8% difference observed between day and night-time stem-$CH_4$ emissions from both young and mature *Alnus glutinosa* (Figs. 3.2 and 4.4; Table 3.3) suggests diffusion-driven transport is the dominant transport mechanism. However, the possibility of a small contribution from an additional transport mechanism (convective and/or transpiration-driven) cannot be ruled

out. A greater contribution from an additional transport mechanism existing alongside the diffusion-driven mechanism was evident from the diurnal patterns in stem-$CH_4$ emissions from *Betula pubescens*, i.e., a 36.4% difference between day and night-time stem-$CH_4$ fluxes observed in mature *Betula pubescens* as opposed to a 13.8% difference in mature *Alnus glutinosa* (Fig. 4.4a). The change from a small flux at night to a relatively large flux during the day observed from *Betula pubescens* strongly indicates a switch from diffusion-driven transport mechanism alone at night to a combination of diffusion, convective and transpiration-driven $CH_4$ transport during the day (Kim *et al.,* 1998). The smaller difference between day and night-time stem-$CH_4$ emissions from *Betula pubescens* in autumn, when compared to summer, supports the hypothesis of two or more transport mechanisms (Fig. 4.4b), whereas in autumn the autumnal leaf loss affected the contribution of convective or transpiration mechanisms, and as a result stem-$CH_4$ emissions and the difference between day and night emissions both decreased.

While diffusion-driven $CH_4$ transport appears to dominate tree-mediated $CH_4$ transport and varying contributions from other $CH_4$ transport mechanisms appear to drive species-specific differences, mesocosm and *in situ* measurements also suggest that tree-mediated $CH_4$ transport mechanisms are influenced by physiological parameters, development stage of the tree, and abiotic factors. For instance, the relationship between stem-$CH_4$ flux and physiological parameters (stem lenticel density in mesocosm experiment, wood specific density and stem diameter in tropical forested wetland, and wood specific density in temperate forested wetland; Figs. 3.3, 3.4 and 5.2; Tables 3.5, 3.6, 4.6 and 5.2) suggests a link between tree species traits and $CH_4$ transport mechanisms, with these parameters possibly influencing $CH_4$ movement into, within, and out of the tree. Wood specific density in particular is an indirect measure of the pore spaces and relative amount of aerenchyma in tree stems and therefore a likely proxy for the capacity of $CH_4$ movement

into and through the tree. These relationships are not static and change with tree developmental stage, resulting in variations in stem-$CH_4$ emission rate. Such an influence could be a straightforward explanation for the observed difference in orders of magnitude between stem-$CH_4$ emissions from young and mature trees in temperate forested wetland. The increased suberization of the roots and of stem surfaces in mature trees compared to young trees may have reduced the capacity of $CH_4$ transport through such trees.

If transport mechanisms were independent of abiotic factors, no change in stem-$CH_4$ emissions between day and night should have occurred in autumn (after leaf loss) in both tree species; instead, a small but continued difference was observed. This difference could be due to various abiotic factors (soil, air and stem temperature, PAR, humidity and wind speed) influencing $CH_4$ production and transport mechanism (Armstrong, 1979; Megonigal *et al.,* 2004). Soil temperature during the diurnal variation experiment varied little in both temperate forested wetland and mesocosms, and therefore displayed no strong relationship with stem-$CH_4$ flux. However, variations in air temperature and PAR displayed a weak yet positive relationship with stem-$CH_4$ emissions from both tree species in temperate forested wetland, suggesting either a direct influence on stem-$CH_4$ emissions (rates of gas diffusion) or indirect influence by regulating soil $CH_4$ production (Hosono & Nouchi, 1997; Macdonald *et al.,* 1998; van Winden *et al.,* 2012). Air temperature and humidity are known to drive pressurised gas transport in many wetland plants (Armstrong *et al.,* 1992, 1996; Graffmann *et al.,* 2008) and may have played a role in driving stem-$CH_4$ emissions.

### 6.5. Obj.4. To identify and characterise key environmental variables affecting tree-mediated $CH_4$ emissions.

Objective 3 was evaluated only in a temperate environment (Chapters 3 and 4). These chapters shed light on the potential global warming feedbacks on tree-mediated $CH_4$ emissions and suggest that increased temperature (Chapter 4) and higher water-table levels (Chapter 3) positively affect tree-mediated $CH_4$ emissions.

Although, water-table depths controlled $CH_4$ production and in turn affected tree-mediated $CH_4$ transport and release in the mesocosm experiment (Chapter 3), water-table variations were not a dominant control on stem-$CH_4$ emission rates *in situ* in the temperate forested wetland (Chapter 4). Water-table depths significantly affected both $CH_4$ production (as demonstrated by lower pore-water $CH_4$ concentration in hollows; Fig. 4.6) and soil emissions (as demonstrated by lower $CH_4$ emissions from non-vegetated hollows; Fig. 4.3; Appendix V and VI). These results demonstrate that soil emissions are more sensitive to water-table fluctuations than stem-$CH_4$ emissions and small changes in water-table depths (< 14.5 cm) may not significantly impact rates of stem-$CH_4$ emissions (discussed further in Chapter 4; section 4.5; Page no: 112). However, large water-table variations that might control $CH_4$ production, $CH_4$ oxidation and ability to transport $CH_4$ by trees due to roots failing to reach the $CH_4$ production zone, could influence rates of stem-$CH_4$ emissions (as observed in LW mesocosms; Chapter 3). Although the influence of water-table depths on tree-mediated $CH_4$ emissions were not measured in tropical forested wetland, results obtained from the temperate forested wetland suggest the influence of water-table depths on stem-$CH_4$ emissions may be greater in SE Asian forested wetland because water-table depth variations are > 15 cm (difference between dry and wet season) and are the principal

control on $CH_4$ production (Jauhiainen *et al.,* 2005) since temperature variations are minimal.

A significant decrease in stem-$CH_4$ emissions with decreasing temperature was observed for *Alnus glutinosa* and *Betula pubescens* possibly due to temperature affecting $CH_4$ production in soil (Bergman *et al.,* 1998; van Winden *et al.,* 2012), substrate quality and availability (Davidson & Janssens, 2006), and $CH_4$ transport through trees (reduced $CH_4$ transport). Notably, rates of stem-$CH_4$ emissions decreased in winter for both tree species in spite of high pore-water $CH_4$ concentrations, suggesting that $CH_4$ transport efficiency decreased with decreasing temperature probably through temperature control of tree physiological parameters (phenological events such as autumnal leaf loss) and $CH_4$ transport mechanisms (cooler temperature decreasing diffusion rates) resulting in reduced stem-$CH_4$ emissions.

A heterogeneous temperature response of $CH_4$ emissions from *Alnus glutinosa* and *Betula pubescens* was observed, with a more pronounced decrease in stem-$CH_4$ flux for *Betula pubescens* than for *Alnus glutinosa* with decreasing temperature. A similar trend was observed when temperature coefficients ($Q_{10}$), i.e., rate of change in a system with a temperature increase of 10 °C, were calculated using equation 6.1 for all $CH_4$ transport pathways (summarised in Table 6.1). The reduced temperature effect on *Alnus glutinosa* when compared to *Betula pubescens* is apparent from their temperature responses. It is likely that a number of mechanisms combined to produce such heterogeneous temperature response and are discussed in Chapter 4 (section 4.5; page no: 107-110). The comparison of $Q_{10}$ coefficients between different $CH_4$ emission pathways also highlights the reduced significance of temperature on stem-$CH_4$ emissions in general when compared to all other $CH_4$ emission pathways.

$$Temperature\ response\ coefficient\ (Q_{10}) = \left(\frac{Y_1}{Y_2}\right)^{\frac{10}{(t_1 - t_2)}} \quad \text{(Equation 6.1)}$$

Where $T_1$ and $T_2$ are the upper and lower limit of the temperature range (°C), and $Y_1$ and $Y_2$ are the $CH_4$ fluxes at $T_1$ and $T_2$, respectively.

Table 6.1: The $Q_{10}$ coefficients for all $CH_4$ emission pathways studied in temperate forested wetland.

| $CH_4$ emission pathways | $Q_{10}$ coefficients |
|---|---|
| Hollows | 10.5 |
| Hummocks | 4.08 |
| Vegetated hollows | 14.6 |
| Vegetated hummocks | 4.68 |
| *Alnus glutinosa* | 1.53 |
| *Betula pubescens* | 3.03 |

## 6.6. Obj.5. To evaluate the role of trees in forested wetland $CH_4$ emissions and establish an ecosystem-scale $CH_4$ budget by quantifying emissions from wetland-adapted trees and soil surface components.

Objective 5 was evaluated in Chapters 4 and 5 and the results presented provide conclusive evidence for the importance of tree-mediated $CH_4$ emissions in both tropical and temperate ecosystems. All $CH_4$ transport pathways were quantified in order to evaluate the role of trees in forested wetland $CH_4$ emissions. Although the two forested wetland sites varied greatly in terms of soil $CH_4$ dynamics, tree-mediated $CH_4$ emissions were found to be significant. Interestingly, these two studies report similar values for tree-mediated $CH_4$

emissions per hectare (5.7 ± 0.6 vs. 6.7 ± 0.7 g $ha^{-1}$ $d^{-1}$; summer emissions from mature trees from temperate forested wetland compared with emissions reported in Chapter 5 for tropical forested wetland; considering only the emissions from the lowermost 3 m of tree) but differ greatly in ecosystem $CH_4$ contributions (8.8-27% vs. 62-87%). This difference was not due to differences in tree density, since they were nearly similar between the two ecosystems (2450 vs. 2689 trees $ha^{-1}$; both young and mature trees in temperate forested wetland vs. only mature trees in tropical forested wetland). Instead, this difference is attributed to the relatively small contributions of non-tree $CH_4$ emission pathways in the tropical forested wetland.

The under-storey of the temperate forested wetland hosted a denser cover of herbaceous plants. These herbaceous plants provide a lower resistance gas transport pathway compared to wetland trees for escape of soil-produced $CH_4$ to the atmosphere, bypassing the aerobic surface layer. With large land surface cover and higher $CH_4$ flux rates, plant-mediated $CH_4$ emissions contributed substantially to ecosystem $CH_4$ flux. However, such under-storey vegetation was absent in the tropical forested wetland. Methane emissions from non-vegetated hollows also contributed significantly to total ecosystem flux in the temperate forested wetland compared to the tropical forested wetland because $CH_4$ oxidation in the temperate forested wetland was limited to the top 5 cm of the soil layer due to upwelling hydrology. In contrast, up to 90% of the soil produced $CH_4$ was oxidised in the top 0-50 cm soil layer in tropical forested wetland, resulting in only small quantities of $CH_4$ being released at the soil surface (Couwenberg *et al.,* 2010). Under such circumstances, the contribution of tree-mediated $CH_4$ transport pathway will exceed other pathways, which was the case in the tropical forested wetland studied.

Notably, the observation of young tree $CH_4$ fluxes exceeding that of mature tree fluxes highlights the possible underestimations of the overall contributions of tree-mediated $CH_4$ emissions estimated in Chapter 5 (tropical forested wetland; Fig. 5.3), since emissions from young trees were not measured in that ecosystem. Furthermore, the two tree species studied in temperate forested wetland although belonging to the same family, Betulaceae, displayed differences in the pattern and magnitude of $CH_4$ emissions. Therefore, while extrapolating tree-mediated $CH_4$ emissions across ecosystems, tree family can only be used as a proxy to identify the presence or absence of tree-mediated $CH_4$ emissions and not to estimate fluxes accurately.

### 6.7. Regional extrapolation

In order to understand the significance of tree-mediated $CH_4$ emissions at a regional and potentially global context, the results of this study were applied to SE Asia. The stem-$CH_4$ emission rates (2.5 to 10.6 mg $CH_4$ $tree^{-1}$ $d^{-1}$) were used to estimate plot level emissions and annual tree emissions from SE Asian forested wetland. Annual $CH_4$ fluxes from SE Asian forested wetland were estimated using emission rates for hollows of 0.5 to 1.32 g $CH_4$ $m^{-2}$ $a^{-1}$ (Jauhiainen *et al.,* 2005) and 0.29 g $CH_4$ $m^{-2}$ $a^{-1}$ from this study. Emissions from hummocks and pneumatophores (surface area of pneumatophores ~ 43.8 $m^2$ $ha^{-1}$) were negligible in comparison to hollows, but were included at a rate of 0.006 g $CH_4$ $m^{-2}$ $a^{-1}$ and 0.005 g $CH_4$ $m^{-2}$ $a^{-1}$, respectively. Annual $CH_4$ emissions from trees in SE Asian forests (Ea) were estimated using the equation:

$$Ea = F \times D \times A \times d \qquad \text{(Equation 6.2)}$$

Where F is the average $CH_4$ emission per tree (2.5 to 10.6 mg $CH_4$ $tree^{-1}$ $d^{-1}$ based upon stem surface area for 3 and 15 m tree heights); D is the density of trees (2689 trees $ha^{-1}$ (Mirmanto, 2010); DBH $\geq$ 7 cm at ~1.3 m); A is the area of SE Asian forests (112,140 $km^2$; Miettinen *et al.,* 2011); and d is the number of $CH_4$ emitting days (244 days; $CH_4$ emissions are assumed to be zero during the dry season (June to September) as $CH_4$ emissions from trees were not measured during this season and water-table drawdown in the dry season in SE Asian forests is known to impact $CH_4$ emissions; Jauhiainen *et al.,* 2005).

The resulting $CH_4$ fluxes from SE Asian forests are small (0.03 to 0.15 Tg $a^{-1}$ and 0.01-0.08 Tg $a^{-1}$ including and excluding tree fluxes, respectively; Jauhiainen *et al.,* 2005) relative to the global $CH_4$ budget (~500-600 Tg $a^{-1}$; Bousquet *et al.,* 2006) because this biome accounts for only ~10% of forested tropical wetlands globally and produces considerably less $CH_4$ at the wetland surface than more nutrient-rich tropical wetland, where soil $CH_4$ is less effectively oxidised. Therefore, it is expected that tree-mediated $CH_4$ emissions have the potential to make a greater contribution in nutrient-rich forested wetlands, such as those found in the Amazon. The potential for tree-mediated $CH_4$ emission contributions from Amazonian wetlands is evaluated below.

### *6.7.1. Potential contributions to Amazonian $CH_4$ emissions*

An empirical regression model was developed and applied to examine the potential contribution of tree-mediated $CH_4$ emissions in Amazonian wetlands, one of the largest areas of tropical forested-wetland globally.

The model employed stem-$CH_4$ emissions as a function of dissolved pore-water $CH_4$ concentrations observed in the mesocosm study (stem $CH_4$ flux between 2-22 cm stem height = 0.0028 (pore-water $CH_4$ concentration at 20 cm soil depth) - 0.258, $R^2$ = 0.47) and

the published pore-water $CH_4$ concentrations for Amazonian wetlands (Bartlett *et al.*, 1988; Koschorreck, 2000) together with our finding of tree stem emission decline with height above the soil surface obtained in tropical forested wetland. The mesocosm study was designed to elucidate the gas transport mechanisms and pathways in *Alnus glutinosa* saplings in soils with artificially high rates of methanogenesis stimulated by enrichment of substrate supply (Chapter 3), which resulted in concentrations of soil $CH_4$ comparable to those measured in the Amazonian wetlands (Bartlett *et al.*, 1988; Koschorreck, 2000).

Stem-$CH_4$ fluxes along the length of tree were estimated using the stem-$CH_4$ fluxes and stem height relationship established in Chapter 5. Both power and linear relationships were used. The average tree diameter at the base in Amazonian floodplain forests (21.5 cm; Wittmann *et al.*, 2006a, b) was used to estimate stem surface area. The relationship between stem height and stem circumference established in Chapter 5 was applied to estimate stem-$CH_4$ emissions. The estimated stem-$CH_4$ emissions ranged between 20.5-3715 mg $CH_4$ $tree^{-1}$ $d^{-1}$. The lowest stem emissions (20.5 mg $CH_4$ $tree^{-1}$ $d^{-1}$) represent tree emissions from the bottom-most 3 m of tree stem where the dissolved $CH_4$ in pore-water is low (175 µmol $l^{-1}$; Koschorreck, 2000) and stem-$CH_4$ emissions display a linear relationship with stem height. The highest tree emissions (3715 mg $tree^{-1}$ $d^{-1}$) represent emissions from 15 m of the tree stem where the dissolved $CH_4$ in pore-water is high (1400 µmol $l^{-1}$; Bartlett *et al.*, 1988), with stem-$CH_4$ emissions from all trees exhibiting a power function relationship with stem height. Total annual tree-mediated $CH_4$ emissions from the Amazon basin were estimated using the extrapolated average $CH_4$ emissions per tree (20.5-3715 mg $CH_4$ $tree^{-1}$ $d^{-1}$;), the density of trees (672 trees $ha^{-1}$; DBH $\geq$ 10 cm; Wittmann *et al.*, 2006a), area of flooded Amazonian basin, the permanently flooded forest and seasonally flooded forest (730,000 $km^2$, 202,800 $km^2$ and 488,800 $km^2$, respectively;

Melack *et al.,* 2004; Hess *et al.,* 2003), and $CH_4$ emitting months (seasonally flooded forests = 4 months and permanently flooded forests = 12 months).

The total annual tree-mediated $CH_4$ emissions from the Amazon basin were estimated to range from 0.15-2.34 Tg $CH_4$ $a^{-1}$ (if only fluxes from the bottom-most 3 m of tree stems are considered) to 1.75 -27.2 Tg $CH_4$ $a^{-1}$ for whole trees (15 m stem height), representing an additional 6-92% of total $CH_4$ emissions estimated from Amazonian wetlands (29.5 Tg $a^{-1}$; Melack *et al.,* 2004), as currently calculated via so-called bottom-up methodologies.

These estimates highlight the significance of tree-mediated $CH_4$ emissions at a regional level and represent a potentially sizeable source of $CH_4$ to the global $CH_4$ budget. However, these estimates are associated with large variations and uncertainty. Therefore, it is critical that we understand the factors and mechanisms controlling stem-$CH_4$ emissions, along with the geographical distribution, before these emission estimates can be upscaled to global level. Moreover, direct measurement of $CH_4$ fluxes from trees within Amazonian wetland is required.

### 6.8. Recommendations for further work

This study sheds light on the variability and controls of tree-mediated $CH_4$ emissions. However, the research area is still in its infancy and there remains scope for further work as detailed below, although the list stated here is not exhaustive.

- The absence of stem-$CH_4$ emissions from a wetland-adapted tree growing in the same ecosystem as those found to emit $CH_4$ highlights the need to quantify tree-mediated $CH_4$ emissions from a wide range of tree species from various ecosystems.

- Although results of this study suggest insignificant $CH_4$ contributions from mature tree leaf surfaces, further studies on mature trees should measure $CH_4$ emissions from leaf surfaces at various canopy heights. The possibility of the lack of $CH_4$ emissions through leaf surfaces due to excessive $CH_4$ oxidation at leaf surfaces should also be verified.
- The relationship observed between stem-$CH_4$ flux and stem height suggests that the entire tree may release $CH_4$, albeit at much lower rates from the higher portions. Direct measurements and confirmation of stem-$CH_4$ emissions from higher portions (> 170 cm above the soil surface) of the tree is essential.
- Heartwood rot is a well-known phenomenon in upland trees and also is observed in *Alnus glutinosa* (Arhipova *et al.,* 2012). Although no visual evidence of heartwood rot was observed in the stem cores extracted from temperate and tropical forested wetland-adapted trees, the influence of heartwood rot on $CH_4$ production and emission should be investigated further.
- A greater understanding of tree-mediated $CH_4$ transport mechanisms and transport efficiency are essential in order to assess the likelihood of $CH_4$ transport in upland trees. Given that one-third of Earth's land surface is forested, a small flux from upland areas could be significant and therefore the possibility merits investigation.
- There was some evidence of tree species-specific temperature-dependence of stem-$CH_4$ emissions. This temperature-dependence may be a consequence of the influence of temperature on primary production, carbon allocation, $CH_4$ production, transport mechanisms and tree physiology and morphology and should be investigated further. An attempt should be made to disentangle these effects to understand the species level controls.

- Much remains to be learnt regarding the species-specific differences in root and stem structure (e.g., quantity of roots at varying soil depths, root air space volume (a proxy for aerenchyma content) and root tissue composition) and their effect on tree-mediated $CH_4$ transport. Further studies should investigate these physiological and morphological traits and how these vary with tree development stage across various ecosystems.
- The factors that affect rates of tree-mediated $CH_4$ emission as a consequence of tree-species effect on $CH_4$ production by microorganisms in wetland soils should be verified, as should the impact of tree-mediated processes on $CH_4$ production.
- This study provides substantial evidence of positive feedback of stem-$CH_4$ emissions to changes in climate (e.g., temperature and water-table depths). The response of tree-mediated $CH_4$ emissions in various ecosystems in a changing environment should be investigated. Furthermore, the mechanisms responsible for inter-seasonal and spatial variability should be elucidated. Studies using a combination of techniques from flux measurements to isotope fractionation analysis would be particularly useful. Isotope analysis and fractionation in particular will also help to identify and understand factors and their interactions that affect stem-$CH_4$ flux.
- This study highlights the dominance of diffusion-driven $CH_4$ transport mechanism in wetland-adapted trees. Observation such as stem-$CH_4$ emissions decreasing with increasing stem height, relationship between stem-$CH_4$ emissions, pore-water $CH_4$ concentrations and stem lenticel density, and presence of aerenchyma, all suggest that $CH_4$ transport occurs in gaseous form. The extent to which soil-produced $CH_4$ also is transported in aqueous form by wetland-trees remains unclear. Such transport may have a significant impact on rates of tree-mediated $CH_4$ flux as trees

have a high transpiration demand. Therefore, the relative significance of tree-mediated $CH_4$ transport and emissions in gaseous and aqueous form merits further investigation.

- Tree-mediated $CH_4$ emissions contributed significantly to ecosystem $CH_4$ flux in both ecosystems. The contributions of wetland trees to emissions of soil-produced $CH_4$ in all climatic zones should be characterised and quantified, along with temporal and spatial variations.
- A better upscaling technique is essential in order to assess the magnitude and distribution of this source at a global level. A technique that attributes observed variability to individual factors and mechanism is essential.

## 6.9. Summary and Conclusions

- Mesocosm experiment and studies conducted *in situ* reveal new evidence for the capacity of trees to mediate export of significant quantities of soil-derived $CH_4$ to the atmosphere. Stem-$CH_4$ emissions were demonstrated to be significant from trees adapted to both tropical and temperate wetland. The $CH_4$ transported through trees were of soil origin and the tree-mediated $CH_4$ emissions decreased with stem height, although results highlight the potential for the entire tree to emit $CH_4$.
- This is the first study to estimate the contribution of trees to total ecosystem $CH_4$ flux from any climatic zone. These estimates from both temperate and tropical forested wetland clearly demonstrate that tree-mediated $CH_4$ emissions contribute significantly to ecosystem $CH_4$ flux and when scaled fully across various ecosystems may help explain observed tropical enhancements in atmospheric $CH_4$.

- The large ecosystem $CH_4$ contributions observed from tropical and temperate wetland trees reinforces the need to include measurements of these $CH_4$ fluxes in emission inventories of forested wetlands. Given that the initial assessment of the potential of tree-mediated $CH_4$ emission pathway is still pending in almost all ecosystems, this study identifies and describes the likelihood of their dominance in other wetland ecosystems. The study also emphasises the need to accurately measure this pathway in other ecosystems before the emission pathway is fully integrated into the ecosystem and global $CH_4$ budget.
- Stem surfaces dominated $CH_4$ egress from wetland-adapted trees and the contributions from leaf surfaces were concluded to be insignificant. Mesocosm experiment results caution against the use of LAI proxy to upscale tree-mediated $CH_4$ emissions from forested wetlands as no relationship was observed between leaf surface area and stem-$CH_4$ emissions from *Alnus glutinosa*.
- The orders of magnitude difference observed in $CH_4$ flux from young and mature trees suggests that the tree development stage is an important factor controlling tree-mediated $CH_4$ emissions.
- Stem-$CH_4$ emissions in temperate forested wetland varied temporally over both short (diurnal) and long (seasonal) periods as a consequence of changes in $CH_4$ transport mechanisms, abiotic and biotic factors.
- Although the abiotic conditions experienced by the two tree species in temperate forested wetland were similar, the stem-$CH_4$ emissions from the two tree species were distinct, with large differences observed in seasonal emissions, diurnal emissions and stem-$CH_4$ emissions along the length of the tree.
- According to diurnal variation measurements at least two mechanisms are responsible for $CH_4$ transport in trees, one dominating when physiological factors

such as transpiration, stomatal conductance and photosynthesis are absent, and a combination of two or more mechanisms when these physiological factors are active, with species-specific difference.

- Variations in physiological parameters such as stem diameter, wood specific density and lenticel density were mainly responsible for inter-species and intra-species differences in stem-$CH_4$ emissions. Pore-water $CH_4$ concentrations were also partly responsible. These results highlight the importance of both above and below ground factors controlling tree-mediated $CH_4$ emissions.
- A species-specific temperature effect on stem-$CH_4$ emissions was observed, although this temperature effect was less pronounced when compared to non-tree $CH_4$ emission pathways and was also reflected in $Q_{10}$ values.
- Stem-$CH_4$ emissions are less sensitive to water-table depth variations than soil $CH_4$ emissions. Small changes in water-table depth did not affect rates of stem-$CH_4$ emissions from both tree species in temperate forested wetland as stem-$CH_4$ emissions from hollows and hummocks were of a similar magnitude.
- Tree-mediated $CH_4$ emissions are not simply a function of the concentration of $CH_4$ dissolved in pore-water and temperature but are far more complex. Several factors such as tree physiology, environmental abiotic conditions and transport mechanisms control tree-mediated $CH_4$ emissions.

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# APPENDICES

**Appendix I:** Results of stepwise multiple regression analysis of stem-$CH_4$ emissions from *Alnus glutinosa* measured at 20-50 cm stem height and all the independent variables measured. Independent variables include soil temperature, PAR, water-table depths, pore-water $CH_4$ concentrations measured in hollows (n = 3) and hummocks (n = 2) at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 cm soil depths.

| | *Alnus glutinosa* | |
|---|---|---|
| | Coefficients | Standard error |
| Adjusted $R^2$ | 0.829 ($P < 0.001$) | |
| Intercept | 12.2 ($P = 0.573$) | 20.7 |
| Ln (Soil temperature) °C | 34.8 ($P = 0.032$) | 13.4 |
| Pore-water concentrations measured at 20 cm soil depth (μmol $CH_4$ $l^{-1}$) | 0.234 ($P = 0.035$) | 0.93 |

**Appendix II:** Results of stepwise multiple regression analysis of stem-$CH_4$ emissions from *Betula pubescens* measured at 20-50 cm stem height and all the independent variables measured. Independent variables include soil temperature, PAR, water-table depths, pore-water $CH_4$ concentrations measured in hollows (n = 3) and hummocks (n = 2) at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 cm soil depths.

| | *Betula pubescens* | |
|---|---|---|
| | Coefficients | Standard error |
| Adjusted $R^2$ | 0.982 (P < 0.0001) | |
| Intercept | 124 ($P = 0.04$) | 29.3 |
| Ln (Soil temperature) °C | 59.7 ($P < 0.0001$) | 7.48 |
| Pore-water concentrations measured at 5 cm soil depth (μmol $CH_4$ $l^{-1}$) | -1.24 ($P < 0.001$) | 0.116 |
| Pore-water concentrations measured at 30 cm soil depth (μmol $CH_4$ $l^{-1}$) | -0.194 ($P = 0.007$) | 0.051 |

**Appendix III:** Results of stepwise multiple regression analysis of $CH_4$ emissions from hollows (non-vegetated) and all the independent variables measured. Independent variables include soil temperature, PAR, water-table depths, pore-water $CH_4$ concentrations measured in hollows (n = 3) at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 cm soil depths.

| | Hollows | |
|---|---|---|
| | Coefficients | Standard error |
| Adjusted $R^2$ | 0.959 ($P < 0.0001$) | |
| Intercept | -975 ($P < 0.001$) | 111 |
| Ln (Soil temperature) °C | 507 ($P < 0.0001$) | 33.3 |
| Pore-water concentrations measured at 15 cm soil depth (μmol $CH_4$ $l^{-1}$) | 0.755 ($P = 0.01$) | 0.225 |

**Appendix IV:** Results of stepwise multiple regression analysis of $CH_4$ emissions from hollows (vegetated) and all the independent variables measured. Independent variables include soil temperature, PAR, water-table depths, pore-water $CH_4$ concentrations measured in hollows (n = 3) at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 cm soil depths.

| | Hollow vegetated | |
|---|---|---|
| | Coefficients | Standard error |
| Adjusted $R^2$ | 0.950 ($P < 0.001$) | |
| Intercept | -948 ($P < 0.001$) | 106 |
| Ln (Soil temperature) °C | 471 ($P < 0.001$) | 52.9 |
| Pore-water concentrations measured at 50 cm soil depth (μmol $CH_4$ $l^{-1}$) | 6.86 ($P = 0.001$) | 1.4 |

**Appendix V:** Results of stepwise multiple regression analysis of $CH_4$ emissions from hummocks (vegetated) and all the independent variables measured. Independent variables include soil temperature, PAR, water-table depths, pore-water $CH_4$ concentrations measured in hummocks (n = 2) at 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 cm soil depths.

| | Hummocks vegetated | |
|---|---|---|
| | Coefficients | Standard error |
| Adjusted $R^2$ | 0.866 ($P < 0.001$) | |
| Intercept | -103 ($P = 0.620$) | 199 |
| Ln (Soil temperature) °C | 299 ($P = 0.001$) | 54.3 |
| Water-table depth (cm) | 25.9 ($P = 0.038$) | 10.4 |

**Appendix VI:** Results of stepwise multiple regression analysis of $CH_4$ emissions from hummocks (non-vegetated) and all the independent variables measured. Independent variables include soil temperature, PAR, water-table depths, pore-water $CH_4$ concentrations measured in hummocks (n = 2) at 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 cm soil depths.

| | Hummocks | |
|---|---|---|
| | Coefficients | Standard error |
| Adjusted $R^2$ | 0.439 ($P < 0.016$) | |
| Intercept | 77.6 ($P = 0.005$) | 20.8 |
| Water-table depth (cm) | 5.97 ($P = 0.016$) | 2.01 |

**Appendix VII:** Results of multiple regression analysis of stem-$CH_4$ fluxes from *Alnus glutinosa* measured at three stem heights (20-50 cm, 60-90 cm and 100-130 cm above the soil surface), stem diameter and wood specific density measured at corresponding stem heights, and pore-water $CH_4$ concentrations measured at 20-30 cm soil depths within 1 m radius of the trees under investigation.

| | 20-50 cm | | 60-90 cm | | 100-130 cm | |
|---|---|---|---|---|---|---|
| | Coefficients | Standard error | Coefficients | Standard error | Coefficients | Standard error |
| Adjusted $R^2$ | 0.741 ($P < 0.0001$) | | 0.671 ($P < 0.001$) | | 0.454 ($P = 0.002$) | |
| Intercept | 225 ($P < 0.001$) | 31.6 | 152 ($P < 0.001$) | 23 | 143 ($P < 0.001$) | 25.4 |
| Wood specific density (g $cm^{-3}$) | -164 ($P = 0.002$) | 45.5 | -94.7 ($P = 0.011$) | 33.1 | -81.5 ($P = 0.042$) | 37.1 |
| Pore-water concentration (µmol $CH_4$ $l^{-1}$) | 0.103 ($P = 0.039$) | 0.046 | 0.136 ($P = 0.002$) | 0.037 | 0.083 ($P = 0.045$) | 0.038 |

**Appendix VIII:** Results of multiple regression analysis of stem-$CH_4$ fluxes from *Betula pubescens* measured at three stem heights (20-50 cm, 60-90 cm and 100-130 cm above the soil surface), stem diameter and wood specific density measured at corresponding stem heights, and pore-water $CH_4$ concentrations measured at 20-30 cm soil depths within 1 m radius of the trees under investigation.

| | 20-50 cm | | 60-90 cm | | 100-130 cm | |
|---|---|---|---|---|---|---|
| | Coefficients | Standard error | Coefficients | Standard error | Coefficients | Standard error |
| Adjusted $R^2$ | 0.67 ($P < 0.0001$) | | 0.657 ($P < 0.001$) | | 0.627 ($P < 0.001$) | |
| Intercept | 250 ($P < 0.001$) | 61 | 169 ($P < 0.0001$) | 40.7 | 117 ($P = 0.002$) | 34.5 |
| Wood specific density (g $cm^{-3}$) | -204 ($P = 0.006$) | 66.9 | -148 ($P = 0.005$) | 48.1 | -89 ($P = 0.024$) | 36.6 |
| Pore-water $CH_4$ concentration (µmol $CH_4$ $l^{-1}$) | 0.290 ($P = 0.004$) | 0.9 | 0.265 ($P < 0.0001$) | 0.055 | 0.199 ($P = 0.001$) | 0.05 |